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CYTOHISTOLOGICAL RESPONSES OF VARIETIES OF AVENA TO 2,4-D[1]

Imy Vincent Holt[2]

The gross aspects of the responses of the cereal grasses to 2,4-dichlorophenoxyacetic acid (2,4-D) have been studied extensively, especially with respect to the relative susceptibility of crop plants. Yields, root suppression, and stalk brittleness have been used as criteria for measuring response. Various abnormalities of plant organs have been correlated with the stage of growth at the time of treatment, and with dosage. However, precise knowledge of the histological responses of apical meristems of grasses to growth regulants is relatively meager.

The present study was undertaken to determine the cytohistological basis for the damage to the Avena inflorescence, and for the reduction of the yield of grain by treatment with 2,4-D at known stages of development.

REVIEW OF PERTINENT LITERATURE

The literature pertaining to growth-regulating substances is extensive and is enlarging rapidly. Skoog's (20) monograph deals with the physiology, chemistry, and agricultural uses of growth regulants. Norman (14), and Norman, Minarik, and Weintraub (15) have published comprehensive reviews of studies on herbicides. Loomis (12), and Mitchell (13) have reviewed the literature on the physiological effects of 2,4-D. The present review will be limited to work on the use of 2,4-D on grasses.

Anderson and Hermansen (1) have described the response of wheat, oats, and barley varieties to 2,4-D applied at four stages of development. Their results showed little difference of susceptibility among the varieties. The stage of development of the plants at the time of spraying, and the dosage, were determining factors in the severity of the damage. The heavier dosages produced the most abnormalities, the greatest decrease in yields, reductions in test weight, and germinating ability. They described the gross morphological abnormalities that occurred after treatment at different stages of development.

Klingman (11) treated wheat, oats, and barley at three different dates with 2,4-D at rates of 1, 2 and 3 pounds acid equivalent per acre. Oats showed less striking responses than barley or wheat. Plants treated at

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[2]The author is indebted to Dr. John E. Sass and Dr. Iver J. Johnson for assistance in planning and conducting this study. Appreciation is also expressed to Dr. R.E. Atkins for procurement of seed and assistance in the field and to Professor Paul G. Homeyer for statistical advice.

the "prejoint" stage gave the most responses. The upper leaves were rolled and stiff and the plants were darker green in color. Significant reductions in yield were obtained in all the treatments with the 3-pound rate applied at the boot stage and at the "jointing" stage. Overland and Rasmussen (17) observed that wheat and barley, treated with 2,4-D at $\frac{1}{2}$- and 1-pound rates at the "early shooting" stage, did not show damage or decrease in yield. The 2-pound rate resulted in morphological injury, or decreased yield, or both.

Olson, et al. (16) studied the sensitivity of wheat and barley at different stages of growth to the n-butyl ester of 2,4-D, at the rate of $\frac{1}{2}$-pound and 12-ounce acid equivalent per acre. The two critical periods in both barley and wheat were the seedling stage when the plants were 1 to 5 inches in height, and the boot stage. Damage was most severe at the latter stage. Conspicuous gross abnormalities were observed in the material treated at early stages of development, and the yield was reduced.

Staniforth (21) studied the gross effects of 2,4-D on meristematic tissues of corn. He found that treatments at the time of tassel formation caused severe inhibition of tassel development. Later treatments had less effect. Applications of 2,4-D one to four days before silk emergence caused severe inhibition of seed setting. He concluded that the inhibitory effects occur during meristematic differentiation.

Rodgers (18) applied 2,4-D to corn at the rate of $\frac{1}{2}$- to 1-pound per acre. He described a stimulation of brace root formation, and a resulting interference with the development of the hypodermis, which were associated with stalk brittleness. Stalk bending, leaf rolling and brace root stimulation occurred after treatment at the 6 to 11 leaf stages. He concluded that spraying corn for weed control should precede the 6 leaf stage, or be applied during the 11 to 16 leaf stage of development.

Derscheid (4) described the responses of barley, treated at four stages of development, and found that: (a) the most susceptible period was the 5-leaf stage (tiller buds inhibited), (b) the 5-leaf to early boot stage was relatively tolerant, (c) the pre-heading to late-heading stage was relatively susceptible, (d) the post-heading period was most resistant. He found that seed viability was not impaired, even by heavy application of 2,4-D.

Williams (26) found that the greatest reduction in yield in oats occurred from treatments applied 33, 38, and 39 days after planting. He suggested that yield reductions are closely associated with the reduction in number of spikelets per panicle and the number of kernels per spikelet.

The cytohistological responses of meristems to growth regulants have been mentioned often in the literature, but concise and detailed information is relatively meager, in comparison with the extensive gross observations. The studies of Eames (5, 6), Hamner and Kraus (9), Beal (2), and other workers (22, 23, 24, 25) are cited here only as examples of critical work on the reactions of organs and tissues of dicotyledonous plants to 2,4-D and the following review of this phase will be limited to the grasses.

Watson (24) found that when Agrostis canina L. was grown in soil that was flooded with a solution of 2,4-D, the growth of the root system was reduced and nodule-like growths developed on the roots. Swollen

areas at the base of the plant contained many root initials. He found that newly formed leaves in the bud contained replacement tissue.

Eames (5) studied the correlation of the severity of 2,4-D injury with the stage ontogeny in Setaria italica (L.) Beauv., and S. viridis (L.) Beauv. The injuries in these grasses were found to be comparable to those in the dicots.

A critical study of the effect of 2,4-D on the cells and tissues of plants must be based on precise knowledge of the normal ontogeny of tissues and organs of the specific plants under investigation. The literature on the normal cytohistology of the grass shoot apex in the vegetative phase, during the initiation of the inflorescence, and during the subsequent development of the floral organs, has been reviewed by Foster (8) and Esau (7).

Bonnett(3) described the transformation of the vegetative apex into an inflorescence and the development of successive orders of branches and floral organs in oats as well as in several other grasses.

Sass and Skogman (19) and Holt (10) have studied the time and manner of initiation of the inflorescence in several perennial grasses.

MATERIALS AND METHODS

Plantings of Andrew and Cherokee oats were made at the Agronomy Farm, Ames, Iowa on April 19, 1952, in accordance with the design used by Williams (49). The test was replicated four times. The plots were 15 feet long, separated by two border rows. The inside row was harvested for yield and the outer row sampled for microscopic study. The plots were space planted, 150 seeds per row instead of the usual rate of 20 grams, and primary kernels were selected to insure a uniform stand.

One pound acid equivalent per acre of the n-butyl ester of 2,4-D was applied 9 days after planting, when the seedlings had emerged. Treatments were applied to successive plots 12, 17, 22, 26, 30, 34, and 38 days after planting. All treatments were made at a rate of 40 gallons per acre, under 30 pounds pressure, with a plot sprayer.

A sample of 10 plants per variety was collected prior to treatment in each sub-plot to determine the stage of development at the time of treatment. An equivalent sample was taken 12 and 24 hours after treatment, and thereafter at four-day intervals until the plants were mature.

The growing points were excised by splitting the fresh sprouts down the center and removing the portions above the node of the largest expanded leaf, and the excised pieces were preserved in a modified Allen-Bouin formula. The tissues were processed in a tertiary-butyl alcohol series and embedded in paraffin. All materials were sectioned at 8-10 microns. Serial microtome sections were stained in safranin-fast green for histological studies. Sections for photomicrography and for projection drawings were stained in hemalum for a few minutes prior to staining in the safranin-fast green.

Median longitudinal sections and serial transverse sections were used for diagnosis.

The plants were harvested for yields on July 11 and 12, 1952. Yields were taken separately on the terminal axis, and on the first and second tillers.

EXPERIMENTAL RESULTS

The Vegetative Shoot Apex in *Avena*

The root-shoot axis during germination

The histology of the oat seedling during germination and emergence from the ground has not been previously described in detail. Cell division begins in the root-shoot axis before the fourth day after planting (Fig. 1), however, most of the enlargement of the embryo to this age is due to cell enlargement.

The emerging radicle is enclosed in an elongated coleorhiza (Fig. 1). Three endogenous seminal roots are present in the embryo. Two of these roots are located on the posterior side of the axis at the scutellar node, and the third root is located on the anterior side of the scutellar node, below the epiblast.

Differentiated vascular elements are evident in the axis seven days after planting. Protophloem and protoxylem are present in the region of the vascular arch, at the coleoptile node, and in the mid-rib of the first leaf (Fig. 2).

The shoot apex of the plumule is a short, rounded dome with two leaf primordia, which were present in the embryo of the mature dormant kernel. The first foliage leaf expands within the emerging coleoptile, but the annular meristem of the second leaf does not completely encircle the stem at this stage.

The third leaf is evident seven days after planting (Figs. 3,4). No cell divisions were observed in the coleoptile prior to emergence from the soil, therefore, growth during this stage is by cell enlargement.

The vascular arch of the mesocotyl is well differentiated and the first tiller primordium is initiated in the axil of the coleoptile by the seventh day (Fig. 3). The vascular bundles of the coleoptile have from two to six protophloem and one to three protoxylem elements at the level of the shoot apex (Fig. 4). The first foliage leaf has three well developed major bundles with well defined protoxylem and protophloem.

Phytomers are laid down at intervals of three to four days in both varieties studied, and continue to be formed until transition to the flowering phase occurs.

The shoot apex at emergence of the plumule.

The coleoptiles of the oat plants in the 1952 plantings emerged above the soil surface 7 to 10 days after planting. A treatment was applied 9 days after planting, when the coleoptiles of approximately 85 per cent of the kernels in the planting had emerged. The shoot apex at this age has a one-layered tunica and a homogeneous corpus. Four foliage leaves are usually present, two of which do not have well developed provascular strands. The fourth leaf is a small protuberance on the side of the apex (Fig. 5).

The first tiller is initiated in the axil of the coleoptile, opposite and below the first foliage leaf (Fig. 3). One tiller leaf is present by this time, and the second tiller is evident as a small protuberance in the axil of the first foliage leaf. Organogeny in the tillers begins with the initiation of the prophyllum, followed soon by the initiation of the first foliage

leaf. The first foliage leaf of a tiller may be formed from either side of the apex. Leaf initiation begins in the second tiller 12 to 16 days after planting.

Soon after emergence, the cells of the outer epidermis and the hypodermal layers of the coleoptile show early signs of deterioration and collapse (Fig. 6). Nine days after planting, the first foliage leaf has three well developed primary vascular bundles, which have from 3 to 4 protophloem elements and from 1 to 4 protoxylem elements at the level of the stem apex. The main bundle in the mid-rib of the second-formed foliage leaf is less developed, and may contain one or more protophloem elements at this stage.

The initiation of successive leaves

The histogenesis of leaves in the shoot apex follows the same pattern of activity described in earlier work, (10, 19). From 6 to 7 foliage leaves comprise the total number of leaves in the mature plant in both varieties. The fifth leaf is initiated at 10 to 12 days, the sixth leaf at 15 to 17 days, and the seventh leaf at 20 to 22 days after planting. The latter leaf is always reduced in size and may or may not differentiate a lamina. The sixth or seventh leaf, commonly referred to as the "flag leaf" or "boot", subtends the internode which becomes the peduncle.

The Initiation and Development of the Inflorescence

The transition phase, which is a critical period in the normal development of the plant, occurred in the present plantings at 17 to 20 days after planting. This phase has been described by previous writers (10, 19), and the same patterns of histogen activity are expressed in the Avena shoot apex, except that the two-layered tunica of Avena is maintained during floral organogeny.

The transition phase in oats is expressed by the development of a long cylindrical axis (Fig. 7) which has as many as 3 to 4 leaf primordia. Leaf initiation terminates and cell division is accelerated in the leaf axils. The primordia produced in these axillary zones are the panicle branches, which will be designated first-order branches (Fig. 8). From 5 to 6 first-order branches are produced on a panicle.

The acropetal initiation of the fifth or sixth panicle branch is followed by the next phase, the period of spikelet initiation (Figs. 9, 41). The initiation of spikelets on the higher orders of branches of the inflorescence proceeds in basipetal order. The initiation of the floral organs begins after the first and second glume primordia are initiated (Fig. 9).

Floral organogeny

Floral organogeny was first evident in material collected 30 days after planting (Figs. 9, 10). The lemma subtending the first floret is initiated in the peripheral layer of the corpus, and further evidence of the formation of the floret primordium occurs immediately above this region. Successive floret primordia are formed in acropetal, distichous order (Figs. 9-12). The organs in the floret are initiated in the following order: lemma, stamens, palea, pistil, and lodicules. From 7 to 8 floret primordia may be formed in a spikelet, however, only the lower three florets produce normal sporocytes.

Fig. 1. Longitudinal section of the root-shoot axis of a four-day old germinating embryo. (15X).

Fig. 2. Longitudinal section of shoot apex. The first foliage leaf is fully expanded. (75X).

Fig. 3. Longitudinal section of a seedling shoot apex seven days after planting. Three leaf primordia are evident. The first tiller is initiated at the base of the first leaf. (75X).

Fig. 4. Cross section of the shoot apex at the level of initiation of the third leaf. Note the presence of protophloem and protoxylem in the first foliage leaf, and procambium in the second leaf. (100X).

Radicle (ra)

Shoot apex (ap)

Coleoptile (col)

Coleorhiza (co)

Epiblast (ep)

Root cap (cp)

First leaf (lf_1)

Second leaf (lf_2)

Third leaf (lf_3)

Tiller (ti)

Vascular arch (ah)

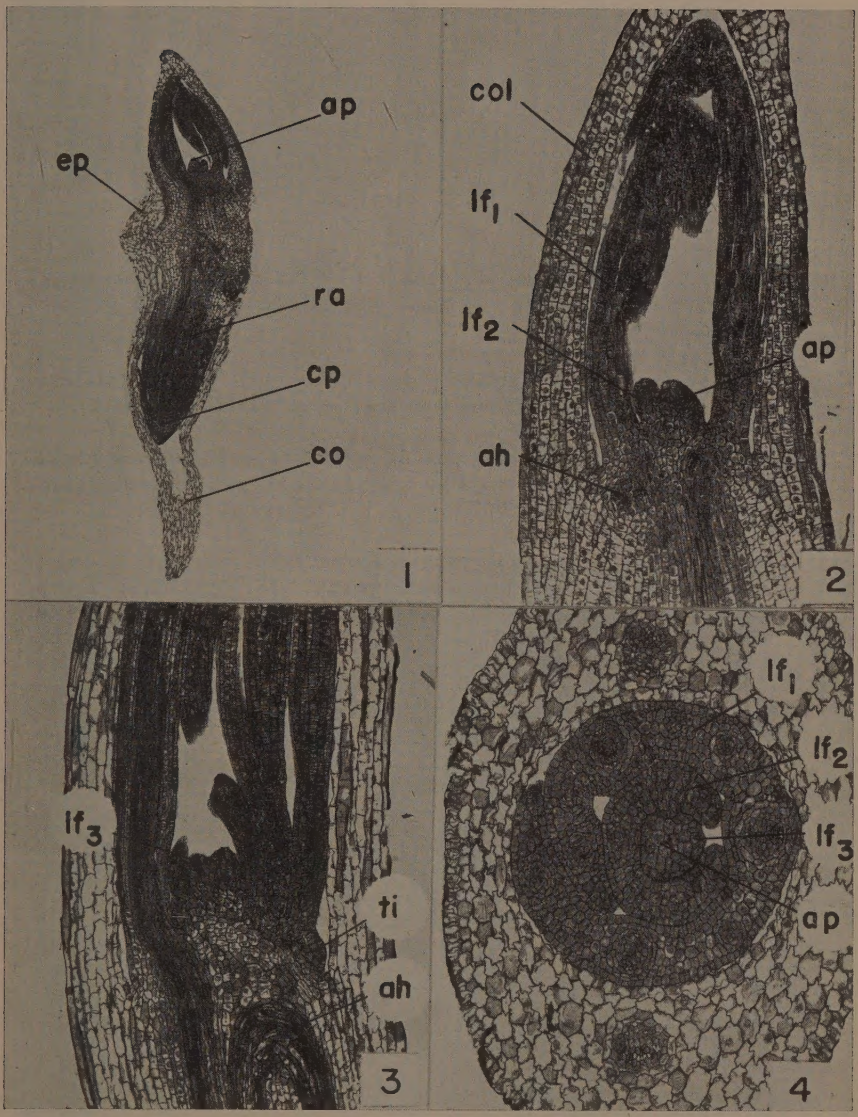
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Fig. 5. Vegetative shoot apex of Avena in longitudinal section at emergence, nine days after planting. The tiller has one leaf primordium. (60X).

Fig. 6. Cross section of vegetative apex, with three leaf primordia. The first 2,4-D treatment was applied at this stage. (60X).

Fig. 7. Longitudinal section of apex at transition to flowering phase. The shoot apex has elongated and has four leaf primordia (compare with Fig. 5). (100X).

Fig. 8. Longitudinal section of young inflorescence with characteristic two-lipped profile of bract and branch. This condition is evident in 17 to 26 day old oat plants. Note the two-layered tunica. (100X).

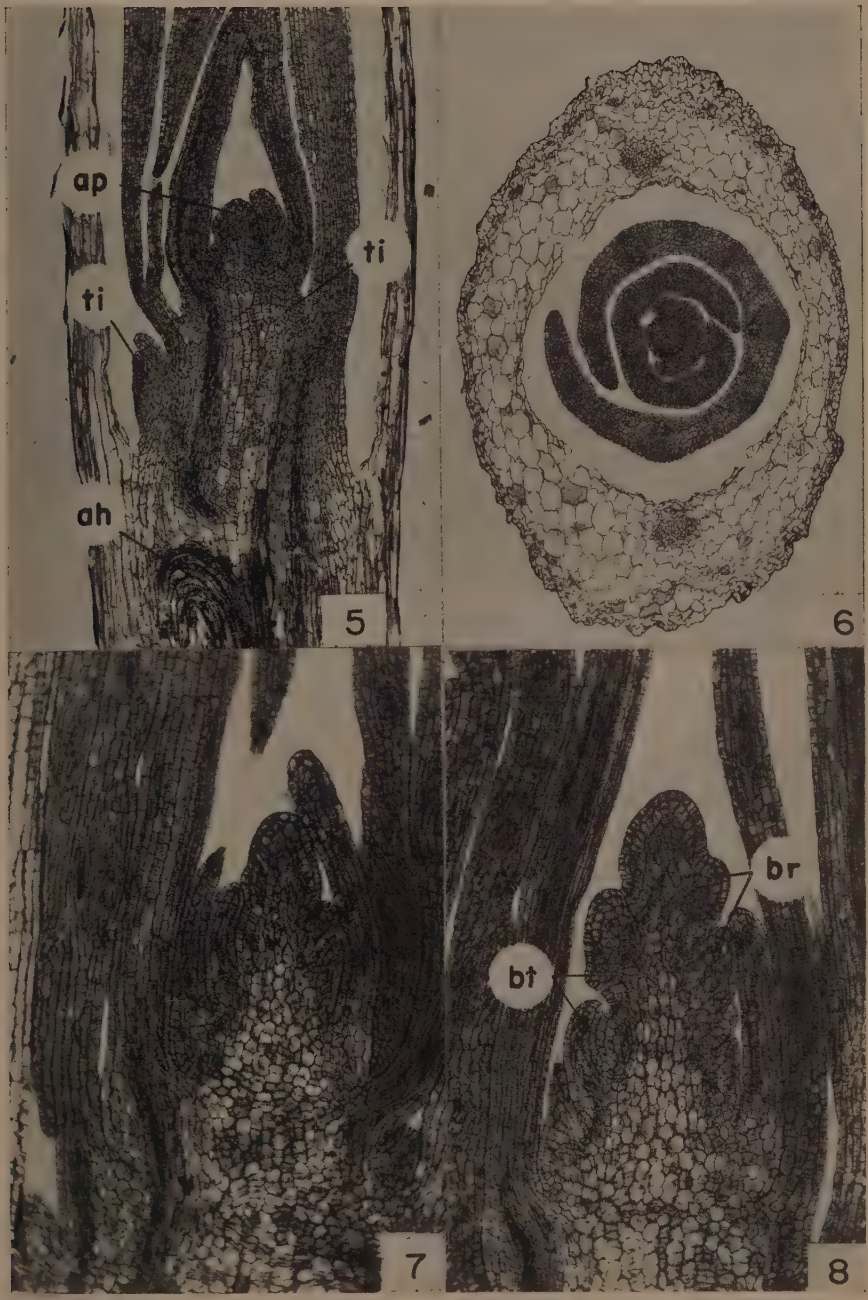
Branch (br)

Bract (bt)

Shoot apex (ap)

Tiller (ti)

Vascular arch (ah)


ap
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8

Formation of archesporia

The stamen is leaf-like in its derivation from the histogens. The primordium elongates and becomes capitate. Archesporial cells are first initiated at this stage from the peripheral layers of the inner mass of tissue. This occurs in the terminal spikelet of the panicle in plants that are from 34 to 38 days old. At this time the anther begins to flatten and elongate. As the archesporial cells enlarge, four conspicuous anther lobes develop (Fig. 13). The duration of archesporial proliferation and enlargement is very short. The archesporium gives rise to the sporogenous cells, the tapetum, and a hypodermis of one layer of cells. The tapetum, which consists of two layers, is encased in a sterile layer of connective cells, contiguous with the epidermis (Fig. 15). The young sporogenous cells enlarge and divide and form the microsporocytes (Fig. 60).

The initiation and development of the gynoecium lags 3 to 4 days behind stamen development. The carpel is initiated on the side of the pistil primordium, adjacent to the outermost stamen (Figs. 12, 50). The carpel is foliar in its ontogeny and grows rapidly along the margins. The residual central dome becomes elevated (Fig. 70) and bent to one side, and becomes the anatropous ovule. The carpel soon encloses the ovule by rapid growth along the margins (Fig. 15).

The first evidence of the archesporium is the enlargement of the nucleus of one of the cells in the peripheral layer of the corpus (Fig. 15). This cell continues to enlarge and becomes the single megasporocyte (Figs. 64, 65).

The Response of the Oat Plant to 2,4-D

Gross responses

Both varieties of oats used in this study show a similar reduction in yield after treatment with 2,4-D (Fig. 59). Yields were reduced in both varieties after treatments applied from 9 to 38 days after planting (Table 1). Treatment at 38 days produced the lowest yields. With subsequent treatments, reduction of yield decreased at each successive treatment. The analysis of variance of yields showed a highly significant difference between treatments and checks, and a significant difference between varieties in yields at successive treatments.

Histological responses of main axis

No cytohistological response is evident in any part of the seedling 12 hours after treatment. The earliest observable response is increased cell division in the corpus and in the leaf primordia, observable 24 hours after treatment.

The response of the histogens in the shoot apex of the main axis is evident four to eight days after treatment. Abnormal leaves are initiated after a brief period of inhibited organogeny (Fig. 27). The polarity of histogen activity is disturbed, and new zones of activity are initiated at abnormal loci on the shoot apex (Fig. 28). A new locus of leaf initiation may be located at the proper level, but laterally displaced on the apex so that the distichous arrangement is interrupted. Normal polarity is soon restored in the peripheral layer of the corpus. The dome of the

Table 1. Mean yield in bushels per acre and reduction in yield expressed as a per cent of the check of the two oat varieties, untreated and treated at eight dates.

Treatment (days after planting)	Varieties				
	Andrew		Cherokee		
	Bu/A	% of Check	Bu/A	% of Check	Ratio Cherokee/Andrew
No treatment	63.0	100.0	57.6	100.0	1.00
9	60.6	94.6	47.6	82.6	.87
12	51.1	81.1	38.1	66.1	.82
17	46.0	73.0	38.4	66.7	.91
22	43.3	68.7	30.0	52.1	.76
26	45.6	72.4	26.0	45.1	.62
30	33.5	53.2	19.7	34.2	.64
34	29.0	46.0	17.5	30.4	.66
38	14.1	22.4	9.8	17.0	.76

shoot apex becomes flattened and widened at the tip (Fig. 27). A condition similar to transition to floral initiation is evident 8 to 12 days after treatment. The shoot apex becomes longer and wider as a result of many cell divisions in the central mass of the corpus. In contrast with the pattern of phytomer development of untreated plants (Fig. 25), treated material of the same age shows a distorted phytomer pattern (Fig. 27).

At the base of the first internode and immediately above the coleoptile node (Fig. 17), the hypodermal layers of the intercalary meristem proliferate by periclinal cell divisions and produce a cambiform zone (Fig. 18). The centrifugal development of this zone encroaches upon the intervening hypodermal cells, which become crushed. The inner epidermis and hypodermal layers of the coleoptile are ruptured as a result of this activity. The narrow proliferated band of tissue is interrupted at the point of initiation of the axillary branch. The proliferated area is confined to a narrow strip, 100 μ to 140 μ in height. Root primordia are initiated in this mass of tissue (Fig. 19) and become an integral part of the secondary root system, which had been previously initiated at the coleoptile node adjacent to, and below, the vascular arch (Fig. 5). All such roots are inhibited in growth and development, and such a condition contributes to the severe lodging expressed in the mature plants. Such roots may obtain lengths ranging from 1 mm to 8 mm before growth is stopped.

Figs. 9-12. Longitudinal sections which show spikelet initiation in terminal spikelet at 30, 34, 36, and 38 days respectively, in untreated material. (42X).

Fig. 9. Initiation of glumes and the first floret.

Fig. 10. Initiation of the lemma of the second floret.

Fig. 11. Initiation of the stamen and pistil in the first floret and initiation of the third floret primordium.

Fig. 12. Initiation of the stamens and pistil and the fourth floret primordium.

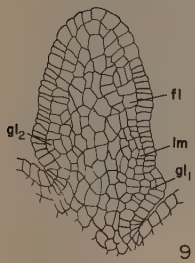
Figs. 13-15. Cross section of the anthers and pistil of the floret. (42X).

Fig. 13. Archesporia formation in the anthers 34 to 38 days following planting. The pistil is a raised dome previous to carpel initiation.

Fig. 14. The tapetum is initiated by periclinal divisions in the peripheral layer of cells surrounding the sporogenous mass 38 days after planting. Note that only one to three sporogenous cells are evident at any one level. The carpel is initiated and partially surrounds the residual mass of meristem which becomes the ovule.

Fig. 15. The tapetum is a single layer of cells which surround the sporogenous mass. The carpel completely encircles the ovule at this age (34 to 42 days following planting). The archesporium is evident in the ovule as an enlarged cell which is derived from the peripheral layer of the corpus. The nucleus continues to enlarge until it becomes from two to three times larger than the nuclei of neighboring cells.

First and second glume (gl_1, gl_2)
Lemma (lm)
First to fourth floret (fl_1, fl_2, fl_3, fl_4)
Pistil (pi)
Stamen (st)
Archesporium (ar)
Tapetum (tp)
Carpel (ca)
Sporogenous tissue (sp)
Ovule (ov)
Anthers (an)



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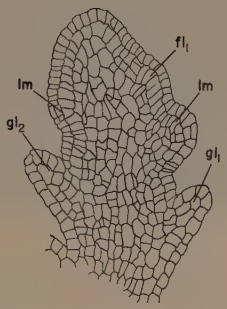

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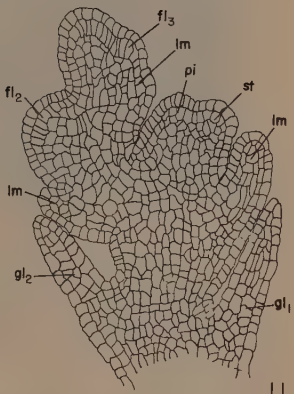

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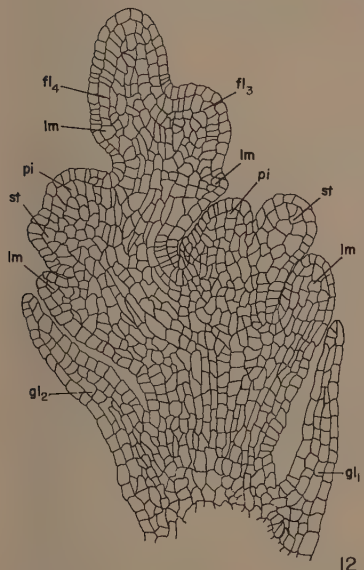

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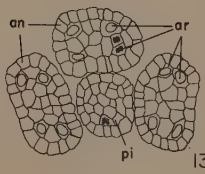

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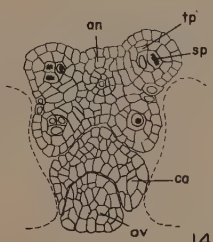

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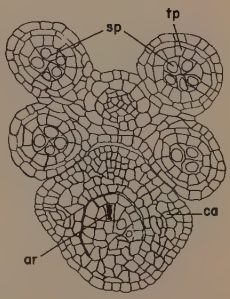

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Fig. 16. Sub-plots of both varieties, Andrew and Cherokee, 20 days following treatment of plants 22 days after planting. Note the characteristic thick, narrow leaves of treated plants and compare with the two rows of the check plants on the extreme right of the picture.

Tiller response

Two distinct types of abnormality can be recognized in the production of inflorescences in the tillers. In the first type, several stem primordia arise at one level in the axil of the first leaf. Each of the axillary primordia produces leaves and a prophyllum. The second type of abnormality is fasciation, produced at the shoot apex during leaf initiation or during the initiation of the inflorescence. In the latter type, the leaves are either common to one axis (Fig. 25) or common to the twin apices (Fig. 24). Multiple organogeny occurs at several other stages of development in treated plants, and is not restricted to this particular treatment.

Three tiller primordia may occur in the axil of the first foliage leaf of plants collected 8 days after treatment. The shoot apices of the tillers are all at comparable stages of leaf initiation, and each shoot has two leaf primordia and a separate prophyllum. Only one or two of the multiple tillers continue to develop, and two apices were observed frequently at maturity (Figs. 23-25).

The flattening of the apex of treated plants is associated with accelerated cell division in the corpus in random planes, induced during the first 24 hours after treatment. Fewer protoxylem and protophloem elements differentiate, the sheath cells do not become fully differentiated, and the bundles remain reduced in size (Fig. 24). Occasionally, in a retarded shoot apex of a tiller (Fig. 22), the last produced leaf may continue to develop to maturity. A normal flowering apex (Fig. 21) affords a comparison with the retarded development of the shoot apices of treated plants shown in Figs. 22-25.

The development of the tillers lags behind that of the shoot apex, from 4 to 7 days in the first tiller and from 5 to 7 days in the second tiller, in the early stages of normal organogeny. The percentage of

Table 2. Number of tillers produced, expressed as a percentage of the total number of plants harvested.

Treatment (days after planting)	Varieties			
	Andrew		Cherokee	
	1st tiller	2nd tiller	1st tiller	2nd tiller
No treatment	59	25	55	24
9	73	46	71	27
12	72	28	68	30
17	73	32	67	40
22	70	32	65	33
26	76	35	70	40
30	74	42	72	42
34	71	24	71	31
38	75	44	65	33

tillers per row was determined in each row for each variety (Table 2). The number of first and second tillers is expressed as per cent of the total number of plants in the row. Treatments did not cause severe suppression of tillering, or pronounced decrease in yield of the tillers. (Table 3).

Table 3. Mean yield in bushels per acre from the main axis, the first tiller and the second tiller respectively.

Treatment (days after planting).	Varieties					
	Andrew			Cherokee		
	M.A.	1st T.	2nd T.	M.A.	1st T.	2nd T.
No Treatment	41.7	14.0	4.6	36.3	13.6	6.4
9	35.5	16.4	8.7	30.2	12.5	4.9
12	28.9	16.6	5.6	23.5	10.7	3.9
17	30.6	11.9	3.5	25.1	8.4	4.7
22	31.0	9.6	2.7	20.0	6.2	2.6
26	28.8	12.3	4.1	17.3	6.2	2.5
30	21.2	8.9	3.5	11.8	5.8	2.2
34	17.7	8.4	3.0	9.3	5.8	2.4
38	7.2	4.5	2.5	5.0	3.7	1.1

Fig. 17. Cross section of normal seedling at the base of first foliage leaf. The tiller primordium has its first leaf primordium. (60X).

Fig. 18. Cross section of plant at the same level as shown in Fig. 18, four to eight days following treatment with 2,4-D. (60X).

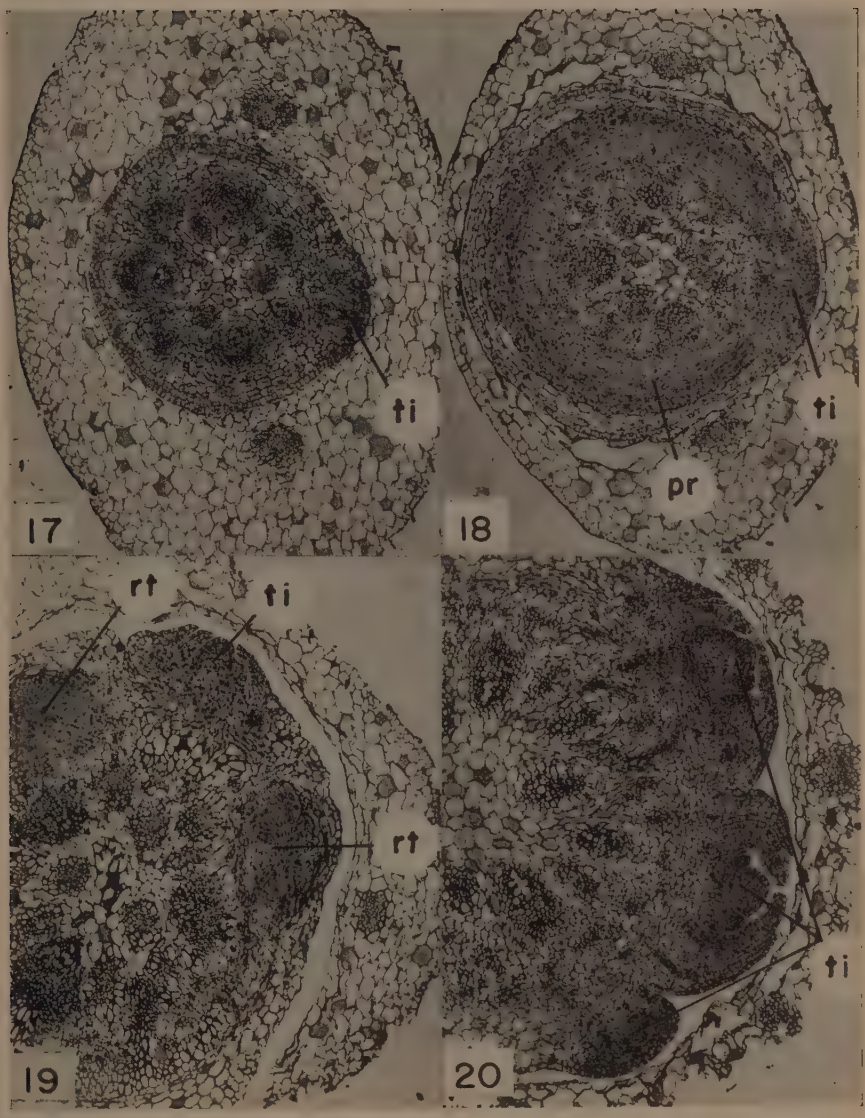
Fig. 19. Cross section of seedling at base of first foliage leaf, showing root primordia. (60X).

Fig. 20. Cross section of stem at level of tiller initiation, in the axil of the first foliage leaf. Three branch primordia are evident, four to eight days after treatment. (60X).

Tiller (ti)

Proliferated tissue (pr)

Root (rt)


ti
17
ti
pr
18
rt
ti
rt
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ti
20

Fig. 21. Longitudinal section of untreated tiller inflorescence, with terminal spikelets on all first-order branches. (15X).

Fig. 22. Longitudinal section of tiller inflorescence, 30 days after treatment. Note that the axillary shoot apex is inhibited whereas the last produced leaf continues to develop. (15X).

Fig. 23. Longitudinal section of twin inflorescences from treated material, 30 days after treatment. (15X).

Fig. 24. Cross section of twin apices derived from a fasciated apex. Note the reduction in size of vascular bundles in the oldest leaves 30 days following treatment. (15X).

Fig. 25. Longitudinal section of twin inflorescence with bilateral symmetry, produced from a fasciated apex. All foliage leaves are common to one axis. (15X).

Fig. 26. Longitudinal section of transition apex in untreated plant, 17 days after planting. Four leaf primordia are evident on the apex. (75X).

Fig. 27. Longitudinal section of shoot apex eight days after treatment, in plants treated nine days after planting. (75X).

Fig. 28. Cross section of shoot apex eight days after treatment. The last two leaves produced on the apex are displaced from the normal position. (75X).

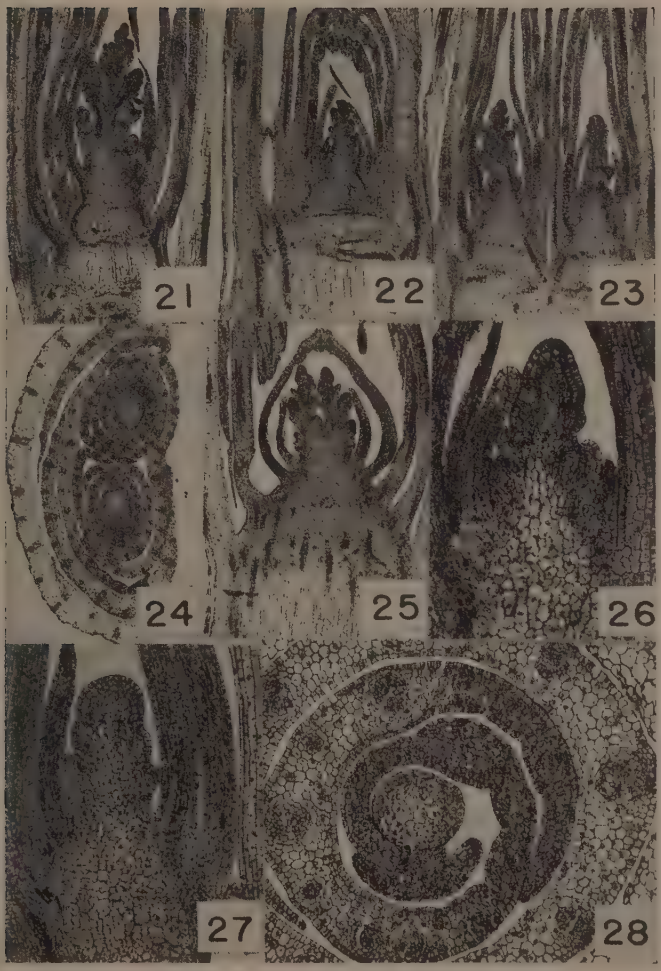
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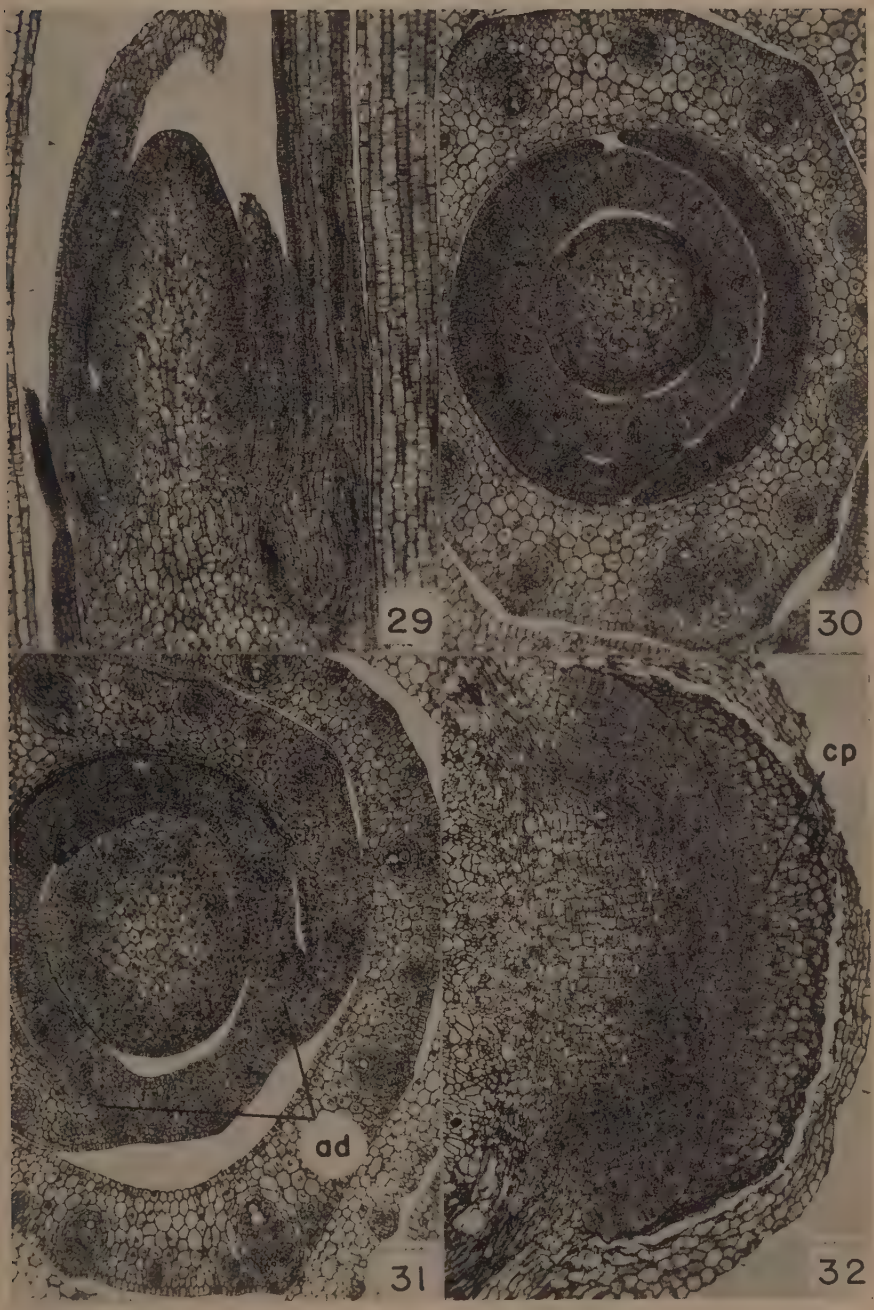
Fig. 29. Shoot apex in longitudinal section, 21 days after treatment, 17 days after planting. The oldest leaf is tubular or sheath-like. (75X).

Fig. 30. Cross section of apex at floral initiation, 30 days after treatment. Two sheath-like leaves have been initiated, with two partial leaves between them. The oldest tubular leaf is the fifth foliage leaf. (75X).

Fig. 31. Cross section of shoot apex 26 days after treatment, showing adnation of two distichously arranged leaves. (75X).

Fig. 32. Cross section of stem at the base of first foliage leaf. Note longitudinal section of fasciated root primordium, and double stele, and continuous calyptrogen over the entire face of the root apex. (75X).

Root cap (cp)


29
30
ad
31
cp
32

Response during leaf initiation

The aberrations of histogen behavior during leaf initiation in treated plants have been described in a previous section. The formation of the "onion leaf" condition and leaf adnation are caused by these changes in histogen activity. The formation of onion leaves, which are sheath-like structures that enclose the shoot apex, occurs most frequently in material treated during the initiation of the fifth and sixth leaf primordia, 12 to 17 days after planting. Proliferation of the annular meristem of the leaf primordium continues uniformly around the rim, and the resulting leaf is a complete sheath (Fig. 30).

Adnation of two leaves is the result of the initiation of two successive leaf primordia at approximately the same level on the shoot apex. The crescent shaped meristems become united in their growth around the apex. The leaf edge may be free on the last-produced leaf (Fig. 31). As a result of this abnormality, the leaf appears to be tubular in mature plants and it is easily confused with the type shown in Fig. 30.

Apices of plants which have this abnormality may show extreme suppression in growth and development. The shoot apex in Fig. 29 shows inhibition of organ initiation 21 days after treatment. Normal plants at this stage have well developed spikelets (Fig. 41).

Response of the shoot apex during the initiation of the inflorescence

During the critical period of floral initiation, the apex is highly responsive to 2,4-D. Leaf and branch initiation are suppressed, or changed in position on the apex. Several days after treatment, the apices show considerable proliferation of the cells of the corpus. Such apices are larger than the normal apex of the same relative age (Figs. 33,34,35). Other apices show very little proliferation at the base (Fig. 36).

The shift in organogeny characteristic of the transition stage may be changed considerably by treatment. The number of first-order branches is reduced in the lower portion of the inflorescence (Figs. 35,37). Leaves are formed without distichous arrangement, and the subterminal branch may surpass the development of the shoot apex (Fig. 34). Such abnormal histogen behavior produces fasciation or multiple inflorescences (Fig. 34).

Severe necrosis occurs in the branch and bract primordia in material collected 20 days after treatment, in plants treated 22 days after planting. The terminal spikelet is initiated, but the final stages of initiation of floral organs is abnormal (Fig. 37).

In plants treated 22 days after planting, some of the first-order branches are completely inhibited (Fig. 49) and spikelet initiation is suppressed (Fig. 42). The same type of response occurs in plants treated 26 days after planting (Fig. 43). Plants treated at 22 and 26 days after planting develop very narrow leaves (Fig. 16).

Response of roots and root-bearing tissues

Fasciated roots are initiated from proliferated hypodermal cells above the nodal plate (Fig. 32). Two or more steles may be initiated in such abnormal roots (Figs. 39,40). The calyptrogen is continuous over the entire face of the root apex, and the root cap is a long narrow envelope enclosing the root apex (Fig. 32). In some roots with a common cortex, the stele may be slightly constricted (Fig. 39) or completely

separated (Fig. 40). The separate steles show few metaxylem elements. Such roots continue to develop for only a very short time. Cell division is stopped when the roots have attained lengths of from one to eight millimeters. Lodging occurs as a result of root suppression.

Response of intercalary meristems

The response of intercalary meristems in the seedling has been described earlier. However, a different type of response was observed in plants treated 26 days after planting (Fig. 44). The internodes are extremely short and the axillary bud is displaced halfway up the internode. Inflorescences are initiated from such axillary branches but do not emerge as mature panicles (Figs. 45-48).

The connective tissues between the hypodermal bundles are poorly developed (Fig. 46), and the inner bundles are scattered at random in the parenchymatous pith. Protoxylem and metaxylem surround the phloem in many bundles. The epidermis and adjacent hypodermal layers are poorly differentiated and are easily damaged. A transverse section of a normal stem at a comparable level in the internode is shown in Fir. 45).

The area below the axillary branch proliferates by a linear series of cell divisions. These cells do not elongate and the internode remains short (Fig. 48). The difference in cell size is marked when compared with the normal stem shown in Fig. 47. The cells of the control material are 85 per cent greater in sectional area than in the treated material.

The lacuna in the treated internode is evident along the axis of the central region, whereas none is evident in the control material (Figs. 46, 47). The lacuna is normally formed below the nodal plate when the internode begins to elongate at the jointing stage.

Response during spikelet initiation

Spikelet initiation in the inflorescence of oats occurs over a relatively long period requiring from 12 to 17 days for the entire panicle. The terminal spikelet of the inflorescence is initiated first, and subsequent spikelet initiation is basipetal. In the present study, this phase began 30 days after planting.

Spikelet abnormalities were observed 20 to 25 days after treatment, on plants that were treated 17 to 22 days after planting (Figs. 50, 51, 52). In some cases, a terminal spikelet initiates a branch primordium in the axil of the first glume, however, the branch fails to develop. The first lemma in the same spikelet may be sterile, without a floret primordium (Fig. 51), whereas floral organogeny is normal in the three successively formed florets.

In material collected 20 days after treatment, 22 days after planting, the terminal spikelets have a sterile lemma, which normally subtends the first floret primordium (Fig. 52). Organogeny in the remaining florets is normal. Low seed yields in these panicles can be attributed to the sum total of all abnormalities induced during development.

In plants treated 30 days after planting (Fig. 41), several types of abnormality were evident. Panicle branches of the fourth-, fifth-, and sixth-order are suppressed in development. The terminal spikelets vary in the degree of development and the degree of suppression of organogeny (Figs. 53-56).

Fig. 33. Longitudinal section of a young oat inflorescence with four panicle branches. Each branch is subtended by a bract. Collected 26 days following planting. The heavy line delimits the tunica and its derivatives. (43X).

Fig. 34. Longitudinal section of a shoot apex 14 days following treatment. The fifth leaf was being initiated when this plant was treated. The three leaves shown were abnormally initiated without the normal distichous arrangement. A subterminal branch is evident to the left of the shoot apex and is subtended by a bract. Compare with Fig. 33. Note the proliferated area in the tunica (43X).

Fig. 35. Longitudinal section of an apex 14 days after treatment showing abnormal leaf and bract initiation. Note the width of the base of the apex and compare it to the control in Fig. 33. Branch initiation is completely inhibited on the right side of the apex. (43X).

Fig. 36. Longitudinal section of an apex 10 days following treatment that shows a narrow base. Branch suppression is evident on the left side of the inflorescence. Compare with the control in Fig. 33. (43X).

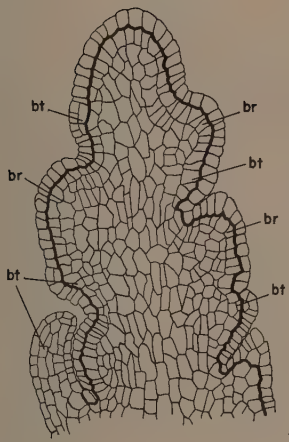
Branch (br)

Bract (bt)

Leaf (lf)

Shoot apex (ap)

Proliferated zone (pr)


bt
br
bt
br
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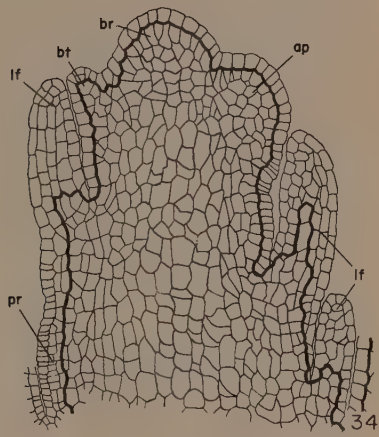
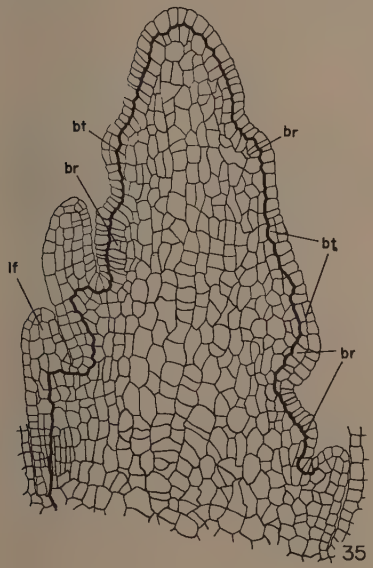
bt

33


br
bt
ap
lf
lf
pr

34


bt
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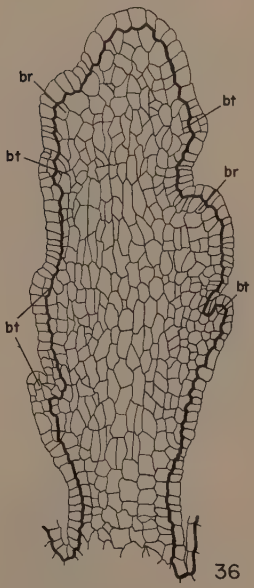
br
bt
lf
br

35


br
bt
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br
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bt

36

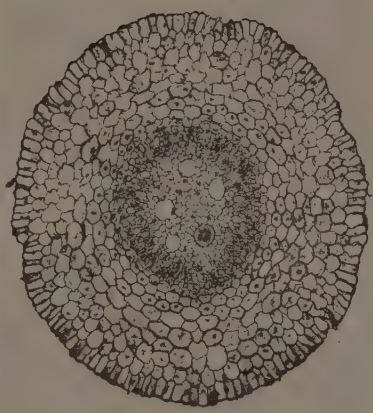
Fig. 37. Longitudinal section of shoot apex during floral initiation, 20 days after treatment, 22 days after planting. The terminal spikelet primordium present, but floral organogeny has been inhibited. (75X).

Fig. 38. Cross section of normal root 150 μ above the root tip. Protophloem, four to five metaxylem elements are evident. (60X).

Fig. 39. Cross section of fasciated root, 150 μ above the root tip. A common cortex is evident. (60X).

Fig. 40. Cross section of fasciated oat root with three separate steles and a common cortex. Each stelar column possesses one or two metaxylem elements. A common root cap overlays the entire root tip. (60X).

37



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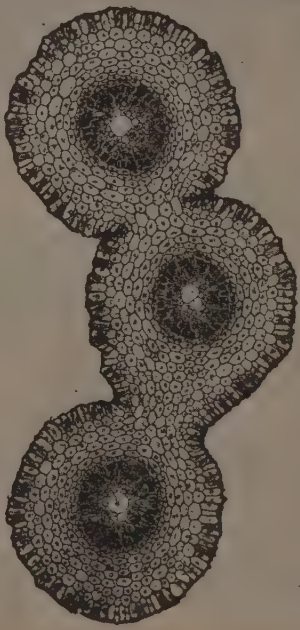


40

Fig. 41. Longitudinal section of young inflorescence at time of floret initiation, 25 to 30 days after planting. (60X).

Fig. 42. Longitudinal section of shoot spex eight days after treatment, 22 days after planting. The initiation of branch primordia is completely inhibited in the lower branches. The terminal portion of the apex shows inhibition of floral initiation. Compare with Fig. 41. (75X).

Fig. 43. Longitudinal section of inflorescence 12 days after treatment, 26 days after planting. Development of lower first-order branches is inhibited. (75X).

Fig. 44. Longitudinal section of shoot apex at transition to flowering phase, 22 days after planting. The area of internode response illustrated in Figs. 47 and 48 is shown outlined. (75X).

First and second glume (gl_1, gl_2)

Lemma (lm)

Floret primordium (fl)

Reduced branch (rb)

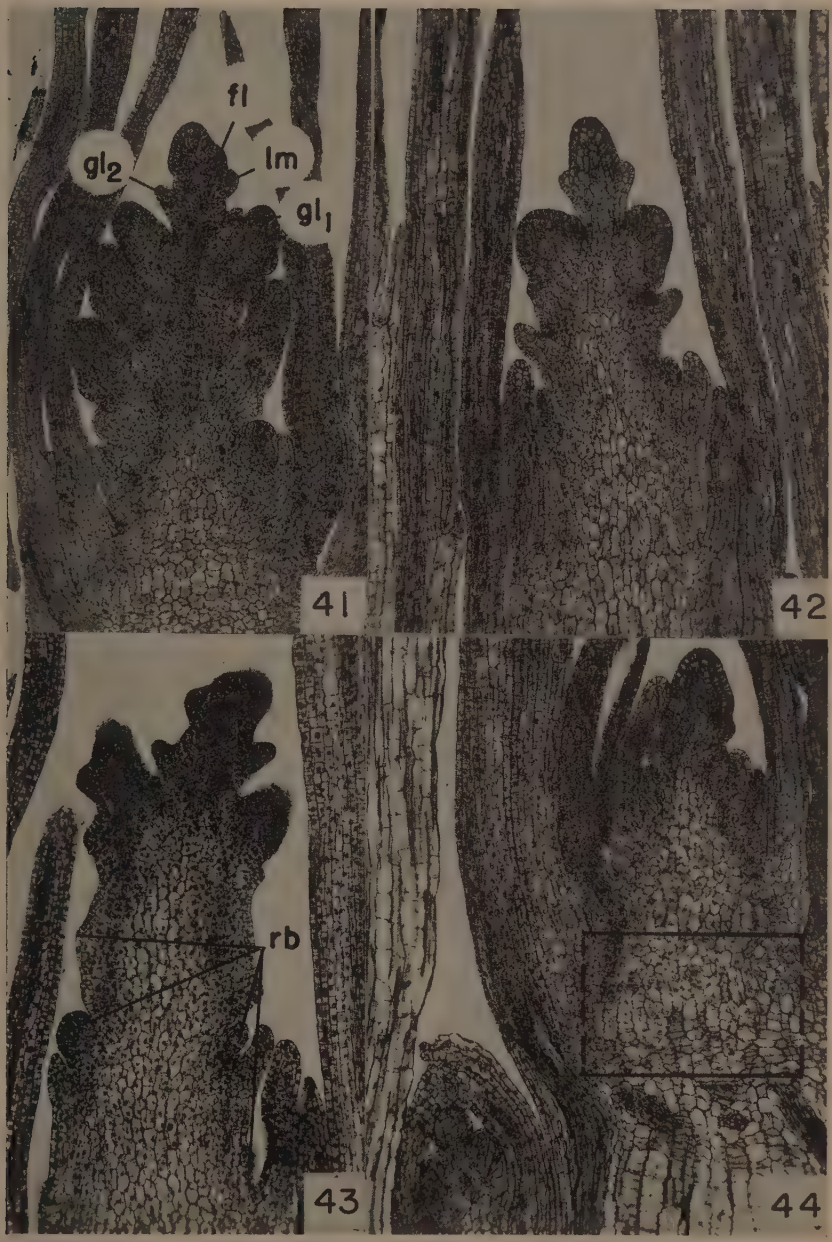
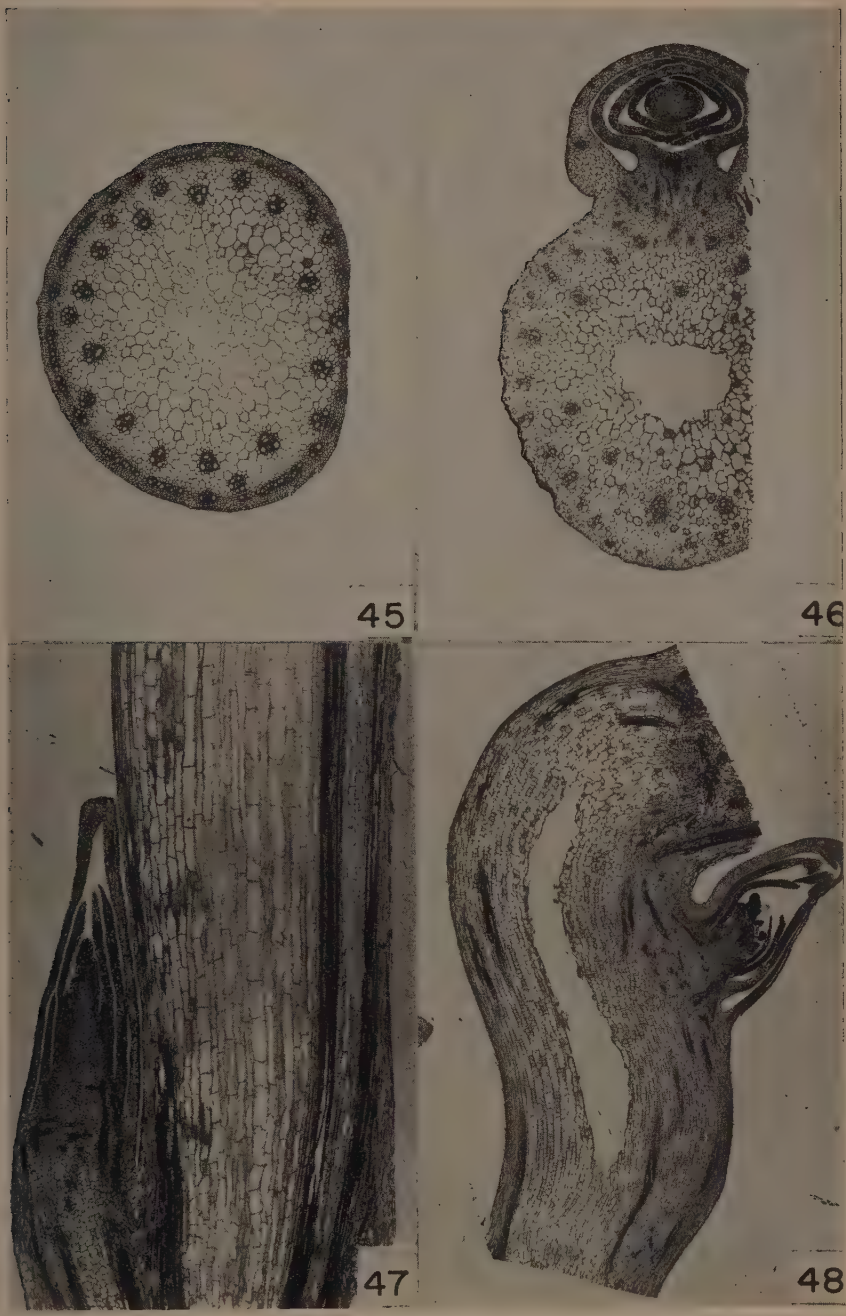

fl
gl2
lm
gl1
41
42
rb
43
44

Fig. 45. Cross section of stem of untreated oats. Note the sclerified tissues between peripheral bundles. The central portion of pith parenchyma disintegrates to form a cavity at maturity. (15X).

Fig. 46. Cross section of stem 12 days after treatment, 26 days after planting. Axillary bud is displaced high up on the internode. Connective tissue between bundles is absent. Epidermis and adjacent hypodermal tissues are poorly developed. (15X).

Fig. 47. Longitudinal section of portion of the internode above second leaf. The axillary branch has a well developed inflorescence. (15X).

Fig. 48. Longitudinal section of abnormal internode above second foliage leaf. The area below the displaced axillary branch has proliferated. (15X).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46
47
48

Fig. 49. Longitudinal section of inflorescence 12 days after treatment, 22 days following planting. The lower branches are suppressed and the shoot apex has broadened by proliferations in the corpus. (75X).

Fig. 50. Longitudinal section of normal spikelet 38 days after planting. (75X).

Fig. 51. Longitudinal section of spikelet 13 days following treatment, 17 days following planting. Note the reduced branch in the axil of the first glume. No floret primordium is produced in the first lemma. Compare with Fig. 50. (75X).

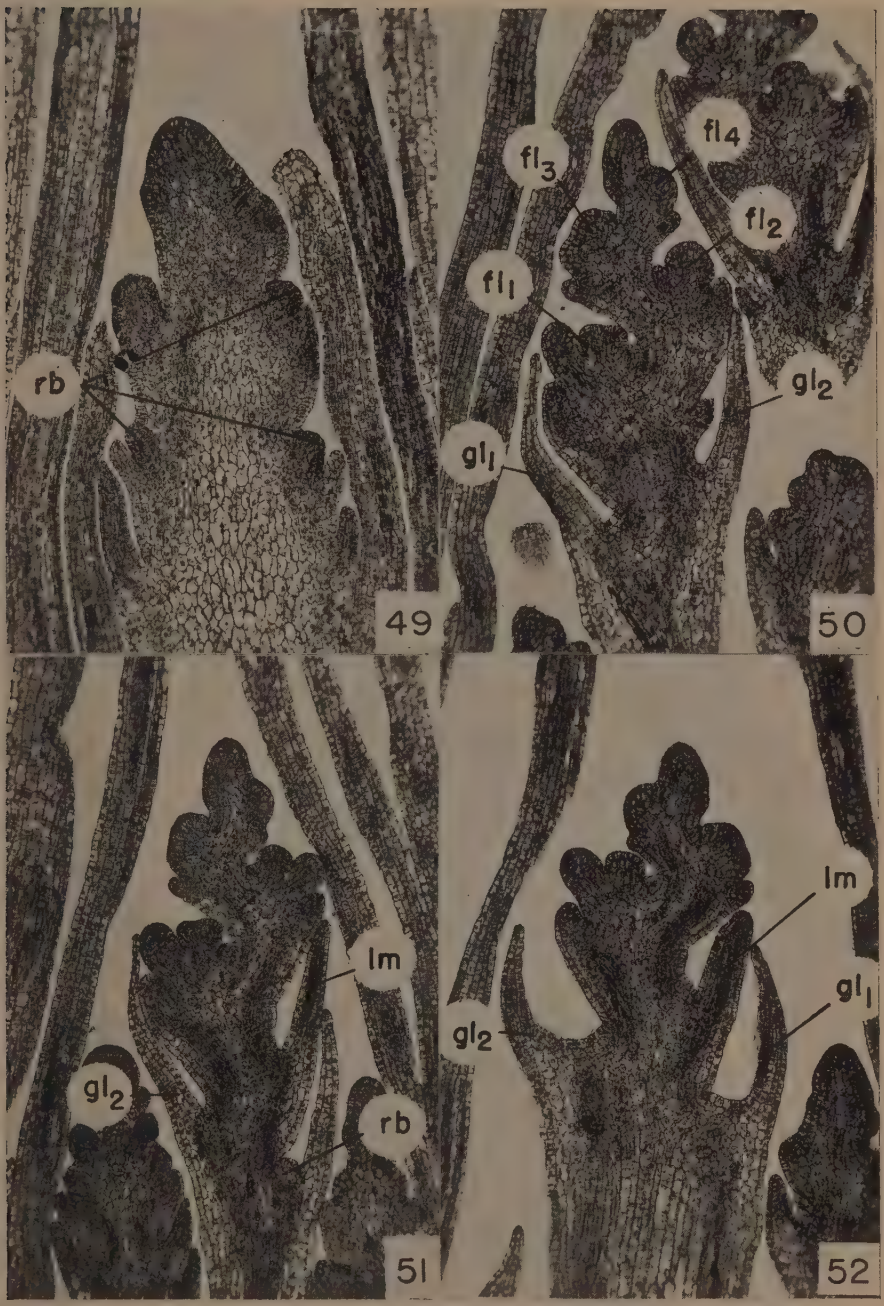
Fig. 52. Longitudinal section of spikelet 20 days following treatment, 22 days after planting. The glumes have a wide axillary furrow. The first lemma is sterile. (75X).

Reduced branches (rb)

First and second glume (gl_1, gl_2)

Lemma (lm)

First to fourth florets (fl_1, fl_2, fl_3, fl_4)


rb
49
fl3
fl4
fl2
fl1
gl2
gl1
50
lm
gl2
rb
51
lm
gl1
gl2
52

Figs. 53-54. Cross sections of the first and second florets respectively, from the same spikelet 17 days after treatment. Plants treated 30 days following planting. (60X).

Fig. 53. Only two anthers are initiated, and pistil primordium is completely suppressed. The second glume, and lemma subtending the first floret are sheath-like and enclose the entire epikelet.

Fig. 54. The first glume and both lemmas are sheath-like and enclose the whole spikelet. Abnormal anthers are formed, one with two vascular bundles, one with five sporogenous columns, and one with normal structure. A double pistil is initiated with two locules, each of which produce a single ovule.

First and second glume (gl_1, gl_2)

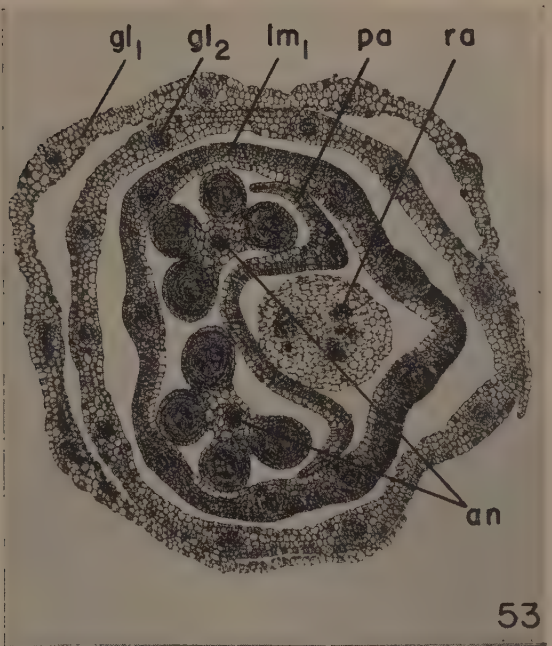
First and second lemma (lm_1, lm_2)

Palea (pa)

Rachilla (ra)

Anthers (an)

Ovule (ov)



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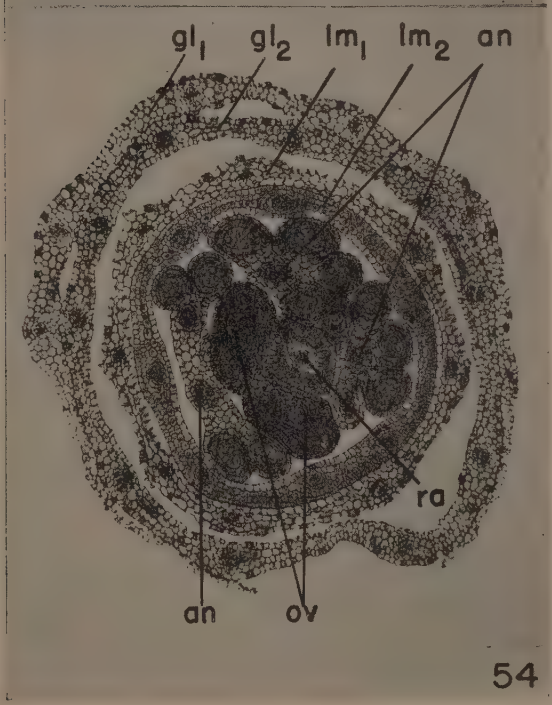

54

Figs. 55-56. Longitudinal sections of inflorescences 12 days after treatment, plants treated 30 days after planting. (60X).

Fig. 55. The inhibition of the initiation and development of the higher orders of branches is evident. The development of the terminal spikelet shows signs of temporary suppression.

Fig. 56. This section shows the inhibition of the initiation of higher orders of branches and of the first glume. Compare with Fig. 50.

Figs. 57-58. Longitudinal sections of terminal spikelet, four and five days after treatment respectively, 34 days after planting. (75X).

Proliferated zone (pr)

Anthers (an)

Pistil (pi)

First and second glume (gl_1, gl_2)

First to third florets (fl_1, fl_2, fl_3)

Reduced branch (rb)


fl1
gl2
gl1
55
fl3
fl2
fl1
gl2
gl1
rb
56
pr
57
pi
pi
an
an
58

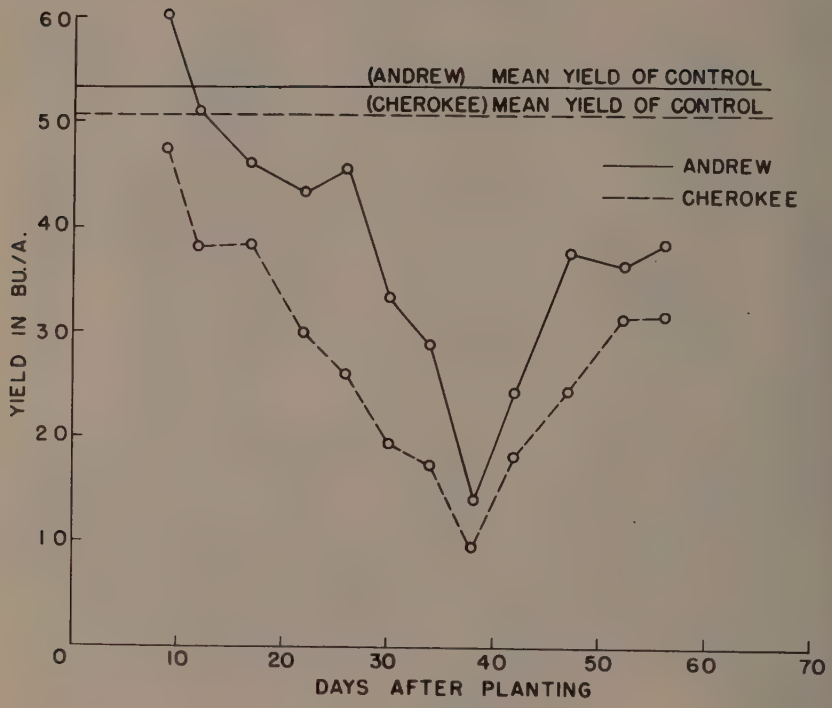

Fig. 59. The trend of mean yields of two oat varieties, Andrew and Cherokee, expressed in bushels per acre at successive treatments in days after planting at 12 stages of growth.

In plants treated 34 and 38 days after planting, a greater number of spikelets are initiated on the successively older terminal branches. With each treatment, after 30 days following planting, more spikelet primordia are suppressed, and ultimate yields are reduced. Fasciated spikelets occur frequently in material treated at 34 days after planting (Figs. 57, 58).

Response during organogeny in the floret

The abnormalities in the floret consist of sheath-like lemmas, reduced organs, and organs with multiple parts (Figs. 53, 54). The glumes subtending the spikelet are also sheath-like and enclose the entire set of florets at maturity. Seeds produced from such abnormal material are light and usually nonfertile. The reduction of floret organs consists of the suppression of the initiation of stamen primordia, the failure of the initiation of the pistil primordium, and failure of the ovule to develop (Figs. 53, 61, 66, 68). Double ovaries with two locules were observed in florets 30 days after planting. Such abnormal organs rarely survive to produce a mature ovule.

Response during archesporial formation

Archesporia are first evident in the anthers 34 to 38 days after planting, and the differentiation of the sporocyte in the ovule occurs four to five days later.

Archesporia are not differentiated in material treated 34 days after planting (Fig. 62, 67). The anther lobes with these deficiencies may remain suppressed (Fig. 67), or continue to produce a leaf-like appendage adnate with the palea primordium (Fig. 62), or produce only one lobe with sporogenous tissue (Figs. 68, 69).

The initiation ot the megasporocyte (Fig. 63) in the ovule is suppressed in material 9 days after treatment, 38 days after planting (Figs. 66, 68, 71). Two different abnormalities give rise to this particular failure. First, the ovule primordium may be suppressed (Figs. 66, 68), and the terminal dome remains as an inert mass of vacuolate cells. The carpel wall, however, continues to develop for a short time and then also aborts.

The second type of abnormality arises in the early stages of the development of the ovule. The peripheral layer of the corpus, from which the single megasporocyte is normally derived, proliferates and produces a bulged dome of necrotic cells (Figs. 70, 71). Ovule abortion follows this proliferation.

DISCUSSION

Previous studies of the responses of cereal grasses to growth regulants have shown that injury occurs in the early stages of growth (1, 4, 11) and that reduction in yield occurs in plants treated during the "early shooting stage" (= floral initiation?) and the "boot stage" (= floret initiation?).

The present study has defined these critical periods of sensitivity in terms of the status of organogeny at specified ages after planting. The correlation of the external stage of development with the stage of organogeny of the shoot apex has provided added diagnostic criteria of practical value in weed control experiments (Table 4).

The reduction in the number of spikelets and in the number of kernels per spikelet has been correlated with yield (26). However, the histological mechanism of the reduction of parts has not been previously described. Branch suppression in the inflorescence gives rise to reduction in the number of spikelets produced in a panicle. The reduction of parts in a spikelet may be caused by a suppression of organ initiation, or to the abortion of an organ, or to severe malformation that prevents development to functional maturity. The reduction in number of kernels per spikelet is associated with the suppression and abortion of the ovary, ovule, or sporocyte.

The stimulation of brace root and adventitious root development has been reported by several workers (18, 24), who showed that proliferation in the cortex-stele interface gives rise to fasciated root primordia, which have a continuous calyptrogen over the entire face of the root apex. Such roots develop several steles within a common cortex. The roots stop growth after cessation of cell division and enlargement and the period of development is very brief. Lodging occurs as a result of inhibition of further root growth.

Fig. 60. Cross section of normal floret, showing sporogenous tissue in anthers, 38 days after planting. (200X).

Fig. 61. Cross section of floret 13 days after treatment, treated at time of anther initiation. One anther was completely suppressed, and cells of ovule are necrotic. (200X).

Fig. 62. Cross section of floret, eight days after treatment. The anther lobes are leaf-like and adnate to the palea at a lower level. Archesporium fail to develop in the leaf-like lobes. (200X).

Fig. 63. Cross section of floret 13 days after treatment, 38 days after planting. The ovule shows two well developed ingeguments and a sporocyte. (200X).

Sporogenous tissue (sp)

Ovule (ov)

Anthers (an)

Megasporocyte (me)

Carpel (ca)

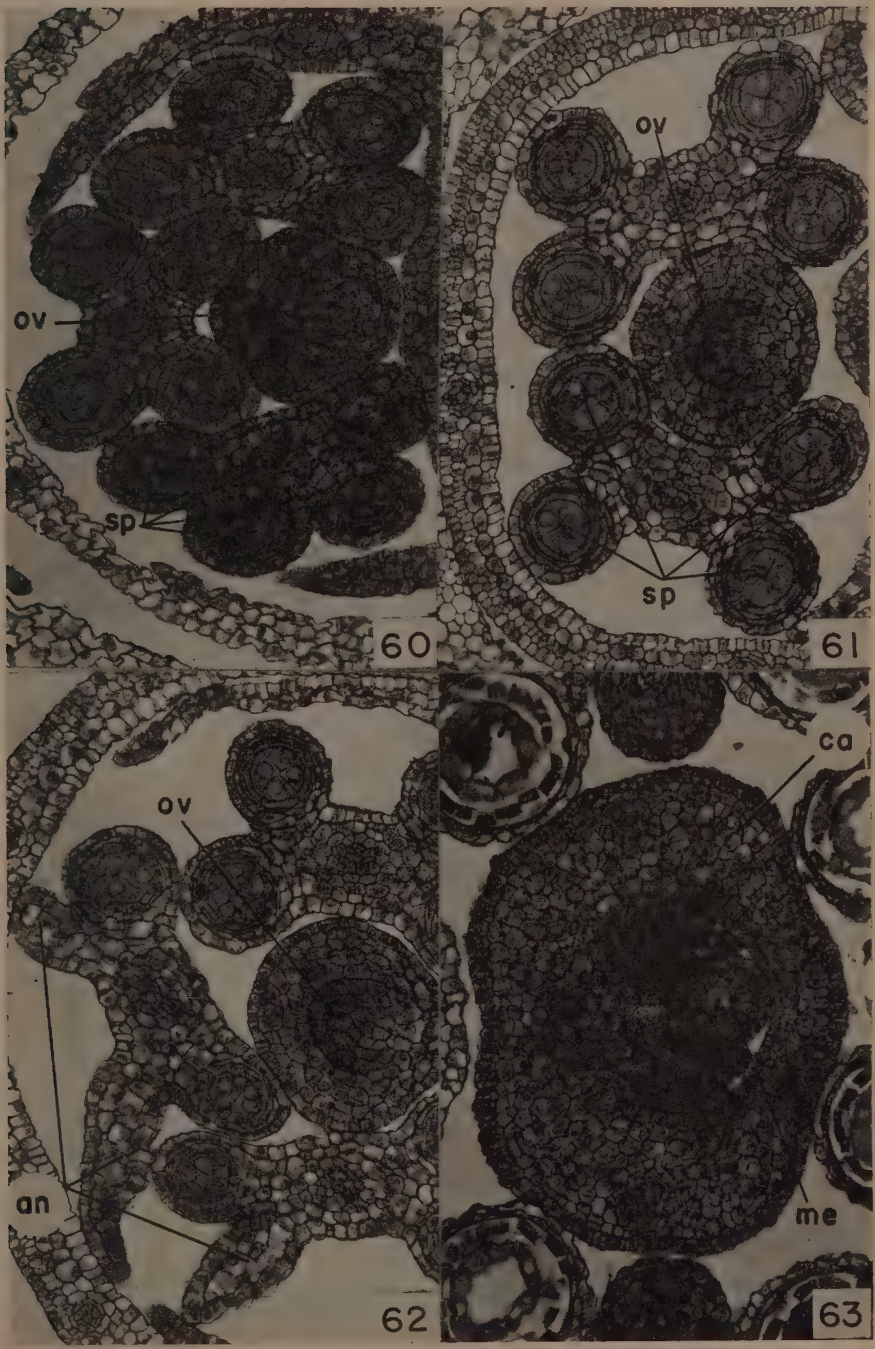
ov
sp
60
ov
sp
61
ov
an
62
ca
me
63

Fig. 64. Longitudinal section of ovary 46 days after planting showing ovule with two integuments, a megasporocyte. (200X)

Fig. 65. Cross section of pistil showing ovule with integuments and sporocyte. Material was collected 13 days following treatment. (200X).

Fig. 66. Longitddinal section through aborting ovule, nine days after treatment, 38 days after planting. (200X).

Fig. 67. Cross section of floret nine days after treatment. Note malformed anthers, with abnormal sporogenous tissue. (200X).

Megasporocyte (me)

Integuments (in)

Carpel (ca)

Ovule (ov)

Reduced anthers (ra)

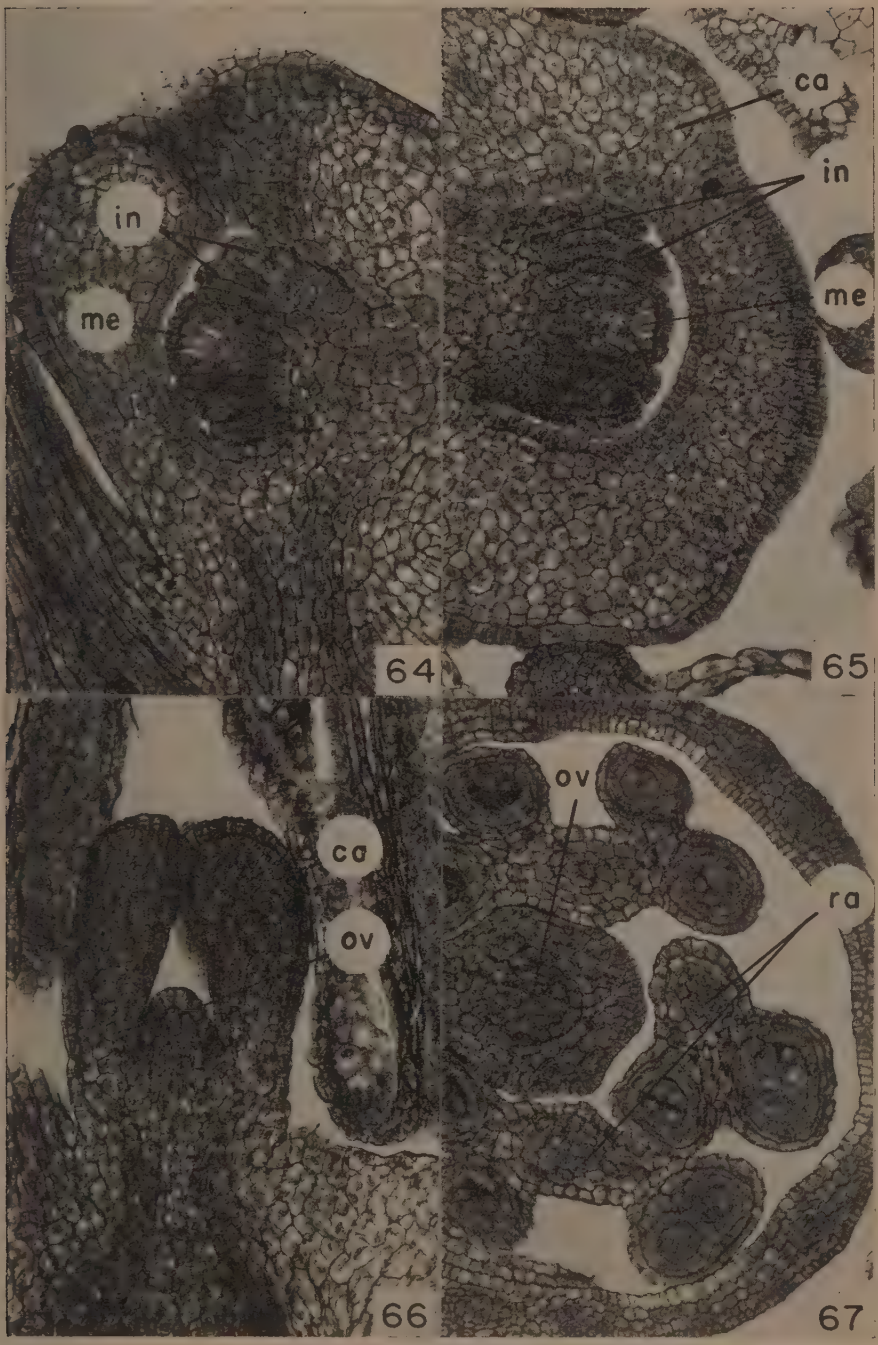
in
me
64
ca
in
me
65
ca
ov
66
ov
ra
67

Fig. 68. Cross section of floret of plant treated 38 days after planting. Anthers are malformed, sporogenous tissue is proliferated. (200X).

Fig. 69. Cross section of floret, nine days after treatment, applied 38 days after planting. (200X).

Fig. 70. Longitudinal section of first floret of spikelet 38 days after planting. Sporogenous cells are present in anther. The lodicule is initiated at the base of and on either side of the filament attachment. (200X).

Fig. 71. Longitudinal section of ovule nine days after treatment, 38 days after planting. Ovule aborts soon after treatment. (200X).

Sporogenous tissue (sp)

Microsporocytes (mi)

Megasporocyte (me)

Anther (an)

Carpel (ca)

Ovule (ov)

Palea (pa)

Lodicules (lo)

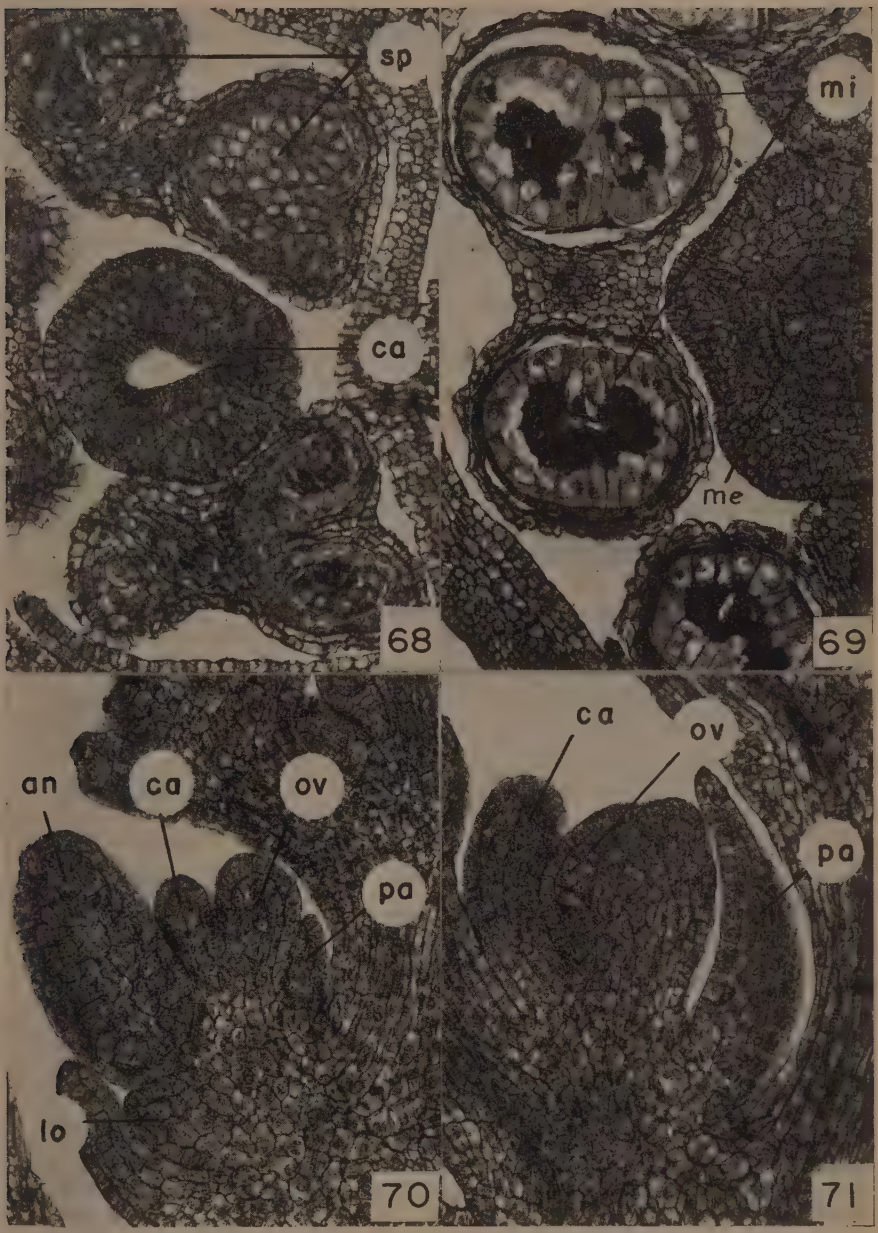
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Table 4. A comparison of stage of normal organogeny of the shoot apex of Avena with the external appearance of the untreated plant at known stages of development.

Days after planting	Organogeny in the shoot apex	External appearance of plants in the field
7	Third foliage leaf primordium	Emergence from soil
9	Fourth foliage leaf primordium	Coleoptile and one foliage leaf
12	Fifth foliage leaf primordium	Two emerged leaves
17	Sixth leaf primordium; elongated shoot apex	Three leaves
22	Seventh leaf primordium; transition to flowering phase	Four leaves; one tiller leaf
26	First-order branches of the panicle	Four leaves; two tiller leaves
30	Spikelet initiation in the terminal apex	Five leaves; two leaves on both tillers evident
34	Second-order branches of the panicle; anthers initiated in the terminal spikelet	Early jointing
38	Floret initiation in upper half of the panicle; initiation of archesporia in anthers and ovule	Six leaves and continued elongation of the plant
42	Floret initiation in panicle branches of higher orders; sporocytes differentiated	Early boot stage; flag leaf evident
47	- - - - - - - -	Panicle emergence from the boot on the main axis
52	- - - - - - - -	Emergence of tiller panicles
56	- - - - - - - -	Panicles completely emerged on main axis and both tillers

Rodgers (18) and other workers (12, 24) have reported that treatment with 2,4-D inhibits the differentiation of the hypodermal layers in the stalk in the lower nodes. In Avena, proliferation in the intercalary meristems above the nodal plate gives rise to a very short internode. The epidermis, hypodermis, and the tissues between the peripheral bundles remain relatively undifferentiated. This accounts for the succulence and brittleness of treated stems.

SUMMARY

A study was made of the histological responses of two varieties of Avena, Andrew and Cherokee, to the n-butyl ester of 2,4-dichlorophenoxyacetic acid. Plants were treated at 12 stages of development.

The developmental histology of the oat plant was studied from germination to emergence above the soil prior to treatment and, subsequently, after each treatment until sporocytes were evident.

The mature kernel of oats has two leaf primordia in the plumule. The third leaf primordium is initiated seven days, and the fourth leaf nine days after planting. Three endogenous seminal roots emerge at the scutellar node during germination.

The inflorescence is initiated from 17 to 20 days after planting. Branch primordia of the first-order branches are evident by 26 days after planting. Spikelet initiation occurs 30 days after planting. The initiation and development of the gynoecium lags behind anther initiation by 3 to 4 days. Archesporia are initiated 34 to 38 days after planting.

Treatments with 2,4-D were begun nine days after planting, when 85 per cent of the plants had emerged through the soil.

The response of both varieties to 2,4-D is similar with respect to the trend in reduction of yield at successive treatments.

Treatments at 9, 12, and 17 days after planting (2, 5, and 19 days after emergence from the soil), induce a change in histogen activity in the shoot apex. Fasciation and abnormal leaf initiation occur. The duration of transition to the flowering phase is prolonged, and floral organogeny is interrupted.

Treatments at 22 and 26 days after planting result in severe necrosis of the first-order branch primordia. Fasciation in the shoot apex gives rise to multiple inflorescences. Spikelet initiation is suppressed in the apical region of the young inflorescence. Tissues of the lower nodes proliferate and produce fasciated roots, which soon abort.

The intercalary meristem proliferates and elevates the axillary branch, which becomes suppressed. Internodal tissues do not differentiate normally and remain thin-walled and soft.

The treatment 30 and 34 days after planting suppresses the initiation at spikelets and the development of branches of higher order. Spikelet fasciation occurs frequently in the treatment 34 days after planting.

The treatment 38 days after planting results in suppression of organogeny in the floret. Malformations and adnation of floret organs occurs during organogeny. The ovule fails to develop in florets which were treated during the initiation of this organ. The initiation of sporocytes is inhibited. The ovule may proliferate and abort.

Tillering was uniform under all treatments, and yield reductions in the tillers results from the same responses as in the main axis.

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EVALUATION OF COMBINING ABILITY IN DACTYLIS GLOMERATA L. I. CLONAL AND OUTCROSS PROGENY PERFORMANCE[1]

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At the present time recombination of high combining clones or lines into superior synthetic varieties appears to offer one of the best opportunities for progress in forage breeding. Paramount to the attainment of this goal, however, is the necessity for forage breeders to develop and utilize efficient procedures for evaluating combining ability. Despite the recent increased tempo of research in forage breeding, there is really little information upon which to base sound judgment in selection of breeding procedures. Concepts utilized in corn breeding have been helpful, but the general physical impossibilities of complete control of pollination on a large scale in most grasses prevents utilization of singlecrosses either for testing combining ability or for commercial hybrids. Other types of outcross progeny tests, such as polycross, topcross, and open-pollination progeny, are more feasible for use. Even with them, lack of adequate pollination control, nonrandom mating, and varying degrees of self-fertilization are suspected to influence progeny performance to unknown extents. Merit of clonal and inbred progeny performance for predicting combining ability, likewise, remains a debatable issue. Principles developed in livestock breeding also may have value in forage breeding because of similarities in respect to highly heterozygous populations, many polygenic characters of major interest from a breeding standpoint, and existence of more or less distinct breeds (ecotypes or even taxonomic species of grasses) with varying adaptive values depending on ultimate utilization. But in livestock, unlike most forage plants, separation of sexes permits breeding control not usually possible in the grasses. Everything considered, the forage breeder must develop his own procedures through intensive investigation, while concurrently utilizing what is possible of principles developed in other related fields. In a large sense the real problem is one of determining how and at what stage of the breeding program to evaluate the range in genetic variability for desired characteristics and the degree to which this range is manifested in the seedling offspring. It is only the heritable variability – considering combining ability as the relative capacity to transmit desirable germplasm to the hybrid offspring – that can be used to effect genetic progress.

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The investigations to be discussed herein represent part of an intensive attempt to study concurrently major aspects of the problem of evaluating combining ability in orchardgrass using the same group of plant material throughout. For this purpose 20 noninbred clones representing a much larger group of selections from long-time stands in Iowa were evaluated for self- and cross-fertility, clonal performance, and singlecross, topcross, and polycross progeny performance. Clones and progenies were evaluated during the same period in adjacent fields and under similar planting conditions to minimize effects of interseasonal, soil fertility, and planting method interactions. Objectives were to determine: (a) potential merits of clonal, singlecross, polycross, and topcross progenies for evaluating combining ability for desirable agronomic characteristics, (b) information of possible predictive value through studies of intercharacter, interannual, and intraseasonal correlations, (c) degree of association among self- and cross-fertility, vigor, and combining ability, (d) estimates of number of replications desired in topcross and polycross nurseries and randomness of pollinations therein, and (e) estimates of general and specific combining ability and their possible utilization. Results pertaining to the first two objectives will be presented and discussed herein, while those relating to the latter three will appear in subsequent articles.

REVIEW OF PERTINENT LITERATURE

Several comprehensive literature reviews pertaining to various aspects of forage breeding have been made in recent years (1, 2, 5, 14, 17, 18, 19, 20, 26). For this reason no attempt at a complete survey need be made here. Rather, attention will be centered on studies particularly related to the development of procedures and principles for evaluating combining ability and to interrelationships of seasons and of agronomic characteristics in regard to the improvement of perennial forage crops.

That combining ability is an inherited characteristic has been amply demonstrated by many studies with corn. Sprague (23) made an extensive review of this work up to 1946 pertaining to combining ability and its evaluation and on inheritance of other important agronomic characteristics. Dearth of the latter type of information in forage grasses was pointed out by Burton (2), who reviewed briefly the relatively few studies made to date on gene action , variability, heritability, and character interrelationships in such species. He stressed the need for further investigations along these lines to facilitate development of superior varieties. Johnson (14) traced the development of concepts for evaluation of combining ability in forages through use of outcross progeny tests and also suggested that poor plot techniques were a possible reason for lack of agreement in studies of the efficiency of various types of progeny. It was emphasized that the most critical need in forage breeding is for additional information on relationships between general combining ability and performance of synthetic varieties. Summaries of problems involved in strain evaluation and in emasculation and pollination were given by Kramer (18) and Keller (16), respectively. They also discussed other investigations pertaining thereto and applications to forage breeding.

Production of singlecrosses, especially on a diallel basis, has long

been considered as the theoretically ideal method for evaluating combining ability not only in corn but also in forage crops. For some time, however, most corn breeders have utilized inbred-variety crosses (topcrosses) for preliminary screening of lines. This practice stems back to results reported by Jenkins and Brunson (11) who found good correlations between the topcross and mean singlecross performance for many characteristics of several groups of inbred lines. More recently, Sprague (23) and others have pointed out that the topcross test primarily provides a measure of general combining ability – average performance in crosses. Several investigators have obtained data, however, to indicate that different varieties or crosses do not always rank lines similarly for combining ability when used as the common parent in top- or test-crosses.

Diallel crossing, paired crossing, and open-pollination, topcross, and polycross progeny testing all have been suggested by various breeders as ways of evaluating breeding behavior in the forages. Advantages and disadvantages of these procedures have been discussed by Johnson (14), Frandsen (5) and Schaepman (21). Of all methods, the polycross test has perhaps received most attention of late. Frandsen (4) was the first to suggest replicated and randomized plantings of many vegetative divisions of clonal selections in such a manner that seed from each propagule of a clone could be bulked for progeny testing. A few years later Tysdal, et al. (26) proposed the term "polycross" for progeny from seed of selected clones (or lines) subjected to outcrossing with other selected clones (or lines) growing in the same nursery. Tysdal and Crandall (25) and Graumann (6) subsequently showed the method practical in the development of superior synthetic varieties of alfalfa. In other recent reports Wellensiek (29) discussed certain theoretical aspects of the polycross test and Schaepman (21) pointed out that a generally high percentage of cross-fertilization, capacity for vegetative propagation, and the long-lived perennial nature of grasses made them ideally suited for use of the polycross test in breeding. The latter also emphasized the desirability of grouping clones according to maturity, bulking seed from different replications on an equal weight basis, and evaluation of clones phenotypically in the nursery used for polycross seed production.

There is evidence accumulating now, however, that polycross testing may not always accurately determine combining ability. Its use is based on the assumption of random intercrossing among all clones in the nursery. Hittle (10) studied separate replicate entries from a polycross nursery of 20 bromegrass clones in comparison with bulk polycross and clonal progenies and obtained evidence of differential pollination from replication to replication. He suggested using a single plant in each replicate and several replications in the polycross nursery to be followed by compositing of seed from all replications for each entry. Gutierrez (7) studied randomness of pollination in polycross nurseries of maize by use of recessive marker genes and polycross yield tests. Both methods gave indication of nonrandom pollination. Certain lines carrying recessive markers showed widely different percentages of outcrossing from that expected with random mating. Number of plants shedding pollen, length of pollen shedding period, and plant height all had some effect on the randomness of mating, while pollen production, location in polycross nursery, and differential cross-compatibility had little or no effect. Stocks simi-

lar in maturity tended to interpollinate more readily than those widely dissimilar, as might have been expected. Wit (30) investigated extent of cross-pollination of perennial ryegrass clones in unreplicated rows and in polycross plantings with the aid of a simple dominant character, roughness of culms and upper leaf sheaths. In the clonal rows, 40 per cent of the fertilization was by adjacent clones, and 74 per cent by three neighboring clones on either side. Cross-pollination decreased rapidly the first three or four rows away and slowly thereafter. More homogeneous pollination was noted in the polycross nurseries, but even there variation in flowering time and pollen production seriously affected homogeneity of pollination.

Comparatively few results on topcross testing in forages are available. As indicated by Schaepman (21), either a single clone or a commercial variety may be used as the common parent. The former would tend to evaluate specific combining ability and the latter general combining ability. Tysdal and Crandall (25) compared polycross, singlecross, and topcross progenies of eight alfalfa clones for efficiency in evaluating combining ability for forage yield and bacterial wilt, cold, and leaf hopper resistance. They found that the various types of progeny tests ranked the clones in a similar way. Corkill (3) compared open-pollination progenies and diallel crosses of five ryegrass clones and noted that the two types of progeny gave 90 per cent similar information on combining ability. His open-pollination progeny seed was produced by isolating the mother clones in an increase field of an improved variety. Thus, the progenies could be considered as topcrosses.

Open-pollination progeny tests have been used for many years with certain forages. In this procedure open-pollinated seed is harvested from the single mother plant, line, or row grown in the breeding nursery. Frandsen (4) found a fairly good relationship between open-pollination progeny and mean singlecross performance in crosses to unrelated testers in a group of timothy clones. In studies with bromegrass and crested wheatgrass Knowles (17) found that open-pollination progeny performance for yield and aphis reaction was highly correlated with mean singlecross performance in the latter species. In bromegrass the correlation was significant for degree of creeping, but not for yield – the latter was considered partly due to presence of inbred plants (resulting from natural selfing) in open-pollination progenies. Polycross progeny yields of the clones showed a poor relationship to singlecross yields in bromegrass. Parental clones and outcross progenies showed little association in performance for yield but significant correlations for spread in bromegrass and aphis reaction in crested wheatgrass. Johnson (13) found open-pollination progeny tests in sweetclover to be effective in the selection of clones high in general combining ability. Subsequent singlecross and polycross tests of derivatives of the material selected for high combining ability in the open-pollination progeny tests showed that the latter had been effective.

The degree of association among replicated clonal nurseries, open-pollination progeny tests, and a singlecross test of a group of orchardgrass clones was investigated by Weiss, et al. (28). Parent-progeny associations for winter survival, leaf width, and panicle number were generally good but those for forage yield were variable and low. Corre-

lations between singlecross and mean open-pollination progeny performance of the two parental clones of each singlecross were low and variable for forage yield, leafiness, and leaf width but high enough for predictive value in the case of panicle number and winter survival. Murphy (20) reviewed a number of papers concerned with comparisons of different types of progeny for evaluating breeding behavior and discussed results of an experiment comparing clonal, self, and polycross progenies of several clones each of orchardgrass, bromegrass, and red fescue. Inbred progenies were space-planted, clonal progenies were planted in wide and close spacings in rows, and polycross progenies were tested in space-planted and drilled rows and in broadcast plots. Average correlations between the same progeny under different planting methods, different progeny and same planting methods, and different progeny and different planting methods all were highly significant. However, correlations involving spaced vs spaced or spaced vs drilled were highest and those involving spaced vs broadcast, or drilled vs broadcast methods were lowest. It was concluded that any of the progeny tests or planting methods offered promise for selecting plants with high yield potential.

Two other factors of considerable importance in forage breeding are interseasonal and intercharacter relationships. Consistency in performance from year to year and lack of undesirable effects of selection for one character on another are highly desirable but not always possible. Tysdal, et al. (26) discussed these factors in regard to alfalfa breeding. They noted that forage production in nurseries was more consistent than seed production; interannual correlations for the former ranged from 0.59 to 0.79 and for the latter from 0.22 to 0.52 in one nursery. In the same studies correlations between forage and seed production ranged from 0.20 to 0.63. In grasses Murphy (20) obtained interannual yield correlations for polycross progenies which were usually highly significant and greater than parent-progeny comparisons. Weiss, et al. (28) noted that orchardgrass clonal nurseries tended to perform more consistently from season to season or from cutting to cutting within seasons than outcross progenies. This was attributed in part to the genotypic consistency in clonal nurseries and lack of it in progeny tests where heterogenity of types and selection pressures would be expressed. These studies were conducted in wide row, spaced, or drilled plantings.

Relationships among important characteristics in orchardgrass have received little study. Schultz (22) investigated the association among several characteristics of 66 S_2 clones. Forage yield was positively and significantly correlated with winterhardiness, plant height and number of culms in the first crop, and negatively and significantly associated with erect plant type, percentage rust infection and number of culms in the second crop. Kalton, et al. (15) in a replicated study of 28 S_0 clones and their S_1 lines grown in a space-planted test obtained correlations ranging from 0.90 to 0.95 between first cutting yield and spring vigor, height, panicle number, or aftermath yield. Higher yielding ability among S_1 lines also was significantly related to low winter injury ($r = -0.80$). Nothing was found in the literature pertaining to intercharacter relationships under solid planting conditions in orchardgrass.

Breeding investigations with orchardgrass have been underway for many years abroad. Early work with this species in Great Britain and

Europe was discussed by Stapledon (24) and Jenkin (12). In this country Schultz (22), Hayes and Schmid (9), Weiss and Mukerji (27), Weiss, et al. (28), Kalton, et al. (15), and Hanson, et al. (8) all have conducted investigations relating to the subject in recent years. Leffel (19) presented a comprehensive review of most of these papers and others pertaining to genetic and cytological studies with Dactylis spp. Consequently, no further mention of them need to be made here.

MATERIALS AND METHODS

Plant material studied in the present investigation consisted of a group of noninbred clones and their singlecross, topcross, and polycross progenies. These clones originated as selections made in 1941 and 1943 by I. J. Johnson of the Iowa Agricultural Experiment Station and M. E. Heath of the Soil Conservation Service in long-time stands in Iowa. Previous studies by Weiss, et al. (28) indicated that differences existed among the clones in agronomic characteristics and performance. In 1948, 20 of these clones were established by vegetative propagation in a space-planted crossing block so that all possible combinations of pairs were obtained. Each clone occurred 19 times. Singlecross seed (reciprocals bulked) of 141 of the possible 171 crosses among 19 clones was obtained by mutual pollinations under bags in 1949. One clone failed to set seed normally in the crossing block and was dropped from the progeny tests. Open-pollinated seed from each location of a clone in the crossing block was harvested and bulked on an equal weight basis and designated as polycross seed. Topcross seed was produced in 1949 in two common-pollinator nurseries where alternate rows were seeded to a commercial lot of orchardgrass. One topcross nursery contained only the 20 clones, while the other contained 80 additional clones of similar origin. Seed from eight spaced, single plant replicates in the two nurseries was bulked on an equal weight basis to give a bulk topcross entry for each clone. Separate lots of seed from each replication also were saved from 12 of the clones for special study.

All outcross progenies were sown broadcast in two adjacent experiments the same fall. Experiment I consisted of 141 singlecrosses, 38 polycrosses (2 entries per clone), and 19 topcrosses. All 198 entries in this test were planted in a randomized complete block design with three replications. Plot size was 4 x 5 feet with timothy borders seeded on the sides and ends of all plots. Experiment II consisted of 8 replicate topcross entries and one polycross and one bulk topcross entry for each of 12 clones for a total of 120 entries. This test was planted in the same manner as Experiment I with replications one, two, and three of the two experiments adjacent to each other in the field. These tests were not harvested in 1950 to permit better stand establishment.

At the same time performance of the 20 parent clones was studied in a tiller bed nursery established in an adjacent field. In this test each plot consisted of 24 propagules planted in three rows of eight propagules each. Propagules were spaced 9-inches apart in 12 inch rows and timothy was seeded as a border on the sides and ends of each plot. Design for this nursery was a randomized complete block with three replications.

Agronomic performance of the clones was studied from 1950 to 1953,

while outcross progeny tests were evaluated in 1951 and 1952. Notes on spring vigor, plant height, leafiness, date of bloom, panicle production, leaf width, and leaf diseases were taken during May and June when appropriate. Some leaf disease readings also were made in August. Two forage harvests were made each year from 1950 to 1952 in the parental test and in 1951 and 1952 in the progeny tests. The first cutting was taken in early to mid-June and the second in late August or September. Seed yields were obtained in the clonal nursery in 1953. Evaluation procedures for each characteristic were as follows:

Early spring vigor score: 1-10, with 1, least vigorous and 10, most vigorous.

Height: height of average panicle in inches from ground level.

Date of bloom: calendar date when 50 per cent or more of panicles were shedding pollen.

Leafiness score: 1-10, with 1, least leafy and 10, most leafy.

Panicle number: actual count of six middle propagules in the center row of each parental plot, and actual count of a 2-foot square area for each progeny plot.

Disease score: 1-4 for parental test, with 1, least disease and 4, most disease; and 1-10 for progeny tests, with 1, least disease and 10, most disease.

Leaf width: 1-10, with 1, narrowest leaf and 10, widest leaf.

Green forage yield: weight of entire plots for parental test. A 3- x 5-foot area was harvested in the progeny tests. All weights were to the closest 0.05 of a pound.

Seed yield: weight of clean seed from entire plots in parental test.

The predominant leaf disease in all tests was brown leaf blight (*Scolecotrichum graminis*), although some leaf and stem rust (*Puccinia* spp.) was evident later in the season each year.

All tests were located at the Agronomy Farm, Ames, Iowa. Plots were fertilized with nitrogen (30-50 lbs. of actual N) early in the spring and again after the first harvest each year. All data collected were analyzed according to standard statistical procedures.

EXPERIMENTAL RESULTS

For simplicity, clonal nursery and progeny test results are presented first and the relationships among them last. For each test agronomic performance, interannual and intraseasonal correlations, and intercharacter correlations are used as a basis for discussion.

Clonal Nursery

Evaluation of parental clones in the tiller bed nursery was made for several reasons. One was to study performance on a basis approaching solid planting to determine if more consistent year-to-year results were possible than if planted in wide-row or space-planted nurseries. Another was to determine if any characteristics exhibited in such a nursery could be used to predict combining ability as measured in outcross progeny tests. A third objective was to study the interrelationships among important forage and other plant characteristics. Use of the tiller bed

nursery for these purposes proved very satisfactory, as stands were excellent throughout the evaluation period from 1950 to 1953.

Agronomic performance

Orchardgrass clones in this test exhibited a wide range in agronomic performance for most characteristics studied. Forage yields were taken twice each year from 1950 to 1952 and statistically analyzed on a green weight basis. Mean differences in yield among clones were highly significant each year and for all years combined. Three-year average yields ranged from 3.50 to 7.57 pounds per plot for the lowest and highest yielding clones, respectively. Cuttings differed significantly in mean yield in 1952 and for the three years combined. The second harvest outyielded the first in 1951, due partly to prolonged cool wet weather throughout the summer. In other years the first harvest yielded considerably more than the second. Average yield of all clones also differed significantly (1 per cent level) from year to year, with highest yields occurring in 1952 – the third year of harvest. Significant interactions of clones x years and clones x cuttings indicated that clones performed differently from harvest to harvest and from year to year. Several clones, however, were consistently high in yield each year.

Panicle production also was measured from 1950 to 1952 and clones differed significantly (1 per cent level) in mean panicle number each year and for all years combined. The clones ranged in mean panicle number for the three years from a low of 29.0 to a high of 114.6 panicles per plot. Mean yearly production of panicles also differed significantly due to a generally much higher production in 1952. The mean square for clones x years was just significant at the 5 per cent level. For further information on potential seed production capacity, the nursery was harvested for seed in 1953. Average seed yield of all clones was 529.3 pounds per acre with a range from 298 to 778 pounds per acre. These yields are high for orchardgrass and indicate a good capacity for seed production. Differences among clones in mean seed yield were highly significant.

Other characteristics evaluated in the tiller bed nursery were: height, date of bloom, spring vigor, leafiness, leaf width, and leaf diseases. The first three characters were studied in 1950 and 1951, the fourth in 1950 and 1952, the fifth in 1951 only, and the last in 1951 and 1952. Except for date of bloom, highly significant differences in mean clonal performance were noted for each characteristic in all analyses. Differences in date of bloom were significant in 1950 but not in 1951 or in the two-year combined analysis. Average dates for the 20 clones for the two years ranged from June 5 to 10, indicating a rather close similarity. The interaction of clones x years was highly significant. Differential winter injury in 1950-51 and not in 1949-50 and delayed flowering due to cool weather in early June of 1951 were partly responsible for this response. Relative differences among clones in leaf disease susceptibility were not consistent in either year. This was due in part to changes in prevalent organisms from June to August. Some clones had relatively low leaf disease scores both years, indicating possible inherent resistance to the disease complex present in the nursery. These results suggest that clones differed genetically in several agronomic traits, but that several

years' data may be necessary to obtain reliable estimates of clonal merit in important respects.

Interannual and intraseasonal correlations

Extent of associations in clonal performance between years, cuttings, readings, and tests for certain traits merit serious consideration in forage breeding. Consistently high relationships between measurements would enable elimination of repeated evaluations during the season or over several years in the same or different tests. Statistical interactions may provide some insight but do not always give desired answers. Consequently, correlations were used for further study of interannual and intraseasonal relationships.

Interannual correlations are summarized in Table 1. Forage yield, panicle number, spring vigor, and leafiness all exhibited a fairly high degree of association in clonal performance between years. Year-to-year interactions for date of bloom and disease score occurred despite the planting method. Plant height differences among clones also varied considerably from 1950 to 1951 judging from the low correlation. This was not indicated in the combined analysis of variance where the clone x year mean square was nonsignificant. As another example, the clone x year interaction for forage yield had a considerably higher F value than that for panicle number. Yet, interannual correlations for the former generally were higher. These results suggest that clone x year interactions, as measured in analyses of variance, do not necessarily indicate the degree of consistency in performance from year to year.

Associations between cuttings or readings within years also are of interest. Table 2 shows that relative capacity of clones to start growth in the spring could be measured any time during May. High yielding clones at the first cutting tended to be high at the second cutting, although the correlation was of low predictive value in 1952. As previously noted, disease response was highly variable from reading to reading.

Table 1. Interannual correlations for agronomic characteristics evaluated in orchardgrass clonal tiller bed nursery from 1950 to 1952 at Ames, Iowa.

Character	Interannual correlations		
	1950-51	1950-52	1951-52
Green forage yield	0.71**	0.69**	0.86**
Panicle number	0.54*	0.70**	0.62**
Date of blooming	0.25	- -	- -
Height	0.43	- -	- -
Spring vigor score	0.65**	- -	- -
Disease score	- -	- -	0.50*
Leafiness score	- -	0.67**	- -

*P < 0.05 ; **P < 0.01 for 18 d.f.

Table 2. Intraseasonal correlations for green forage yields, spring vigor scores, and disease susceptibility scores in orchardgrass clonal tiller bed nursery in 1950, 1951, and 1952.

Item correlated	1950	1951	1952
First and second cutting yields	0.79**	0.72**	0.45*
Spring vigor scores:			
May 2 and May 8, 1951	- -	0.97**	- -
May 2 and May 25, 1951	- -	0.90**	- -
May 8 and May 25, 1951	- -	0.91**	- -
Disease susceptibility scores:			
May 30 and June 15, 1951	- -	0.48*	- -
May 30 and August 5, 1951	- -	0.27	- -
June 15 and August 5, 1951	- -	-0.24	- -
June 4 and August 6, 1952	- -	- -	0.33

*P < 0.05 ; **P < 0.01 for 18 d.f.

All clones included in the present study were evaluated in a replicated space-planted clonal nursery from 1945 to 1947. Degree of association between performance in this earlier test and in the present one provides a measure of the effect of planting method. Correlation of mean results for the two tests were as follows:

Item and year(s) correlated	"r" value
Yield (1945-47) and (1950-52)	0.20
Leaf disease (1947) and (1951-52)	0.36
Bloom date (1946-47) and (1950-51)	0.76**
Leafiness (1946-47) and (1950 and 1952)	0.50*
Leaf width (1946-47) and (1951)	0.58**
Panicle number (1946) and (1950-52)	0.50*

*P < 0.05 ; **P < 0.01 for 18 d.f.

Results show that clonal yield and leaf disease reaction in wide-spaced rows from 1945-47 were not predictive of performance in the tiller bed planting from 1950-52. Dates of bloom were similar in the two nurseries. Correlations for leaf width, leafiness, and panicle number were significant but of rather low predictive value.

Considering all interannual, intraseasonal, and intertest correlations, it is apparent that one year of evaluation was not sufficient to differentiate clonal performance for agronomic characteristics. Wide-spaced planting generally evaluated clones in a manner more or less dissimilar to close-spaced planting, except for bloom date, even though results for bloom date, even though results for several years were obtained in both nurseries. Disease reactions varied both within and between years. Since farm use for orchardgrass is in solid planting (close-drilled or broadcast), it would appear that agronomic evaluation of orchardgrass clones

for most characteristics should be under conditions approaching solid planting and for two years or more in order to obtain reasonably accurate information on clonal behavior.

Intercharacter correlations

Relationship among important plant characteristics is another factor that must be considered in forage breeding, since high forage yield in itself is not always the ultimate criterion of strain superiority. In the clonal nursery spring vigor, leafiness, and panicle number all were highly correlated in a positive manner with forage yield (Table 3). Panicle production and spring vigor likewise were significantly associated, as were leafiness and spring vigor. These correlations were in the desired direction. On the other hand, there was a consistent tendency for later flowering clones to produce fewer panicles and less forage and to start growth later in the spring than earlier blooming clones. Narrow-leafed

Table 3. Correlations among agronomic characteristics evaluated in clonal tiller bed nursery at Ames, Iowa, in 1950, 1951, 1952 and 1953.

	Year	Disease score	Spring vigor score	Height inches	Bloom date (June)	Leafiness score	Panicle no.	Green forage yield
Leaf width score	1951	-0.26	-0.25	0.33	0.52*	-----	-0.58**	-0.15
Disease score	1951	-----	0.47*	-0.23	-0.27	-----	0.39	0.39
	1952	-----	-----	-----	-----	0.25	0.08	0.40
Spring vigor score	1950	-----	-----	0.27	-0.28	0.92**	0.59**	0.95**
	1951	-----	-----	-0.03	-0.63**	-----	0.74**	0.83**
	1950-51	-----	-----	0.17	-0.52*	-----	0.68**	0.92**
Height (inches)	1950	-----	-----	-----	-0.28	0.22	-0.18	0.30
	1951	-----	-----	-----	0.08	-----	-0.04	0.21
	1950-51	-----	-----	-----	-0.04	-----	-0.13	0.25
Bloom date (June)	1950	-----	-----	-----	-----	-0.10	-0.33	-0.16
	1951	-----	-----	-----	-----	-----	-0.69**	-0.38
	1950-51	-----	-----	-----	-----	-----	-0.51*	-0.39
Leafiness score	1950	-----	-----	-----	-----	-----	0.48*	0.94**
	1952	-----	-----	-----	-----	-----	0.16	0.58**
	1950,1952	-----	-----	-----	-----	-----	0.29	0.77**
Panicle number	1950	-----	-----	-----	-----	-----	-----	0.52*
	1951	-----	-----	-----	-----	-----	-----	0.61**
	1952	-----	-----	-----	-----	-----	-----	0.58**
	1950-52	-----	-----	-----	-----	-----	-----	0.62**
Seed yield[1]	1953	0.22	0.18	-0.06	0.00	-0.08	0.30	0.16

*P < 0.05 ; **P < 0.01 for 18 d.f.

1/ Seed yield obtained in 1953 correlated with two- or three averages of clonal performance for other characteristics.

clones generally were highest in panicle production. Leaf disease intensity, height, and seed yield showed little or no relationship to other characters. This would indicate that the tall, disease resistant clones were not necessarily high in forage yield or panicle number, nor were the disease resistant clones with high panicle numbers always high in seed yield. These results suggest that selection of clones exhibiting a combination of rapid, early spring growth, high forage and seed production, leafiness, leaf disease resistance, and late flowering would be complicated mostly by the adverse relationship between date of bloom and other characteristics and the apparent inability to select for high seed production by choice of disease resistant, panicle productive clones.

Outcross Progeny Tests

Outcross progenies of most clones studied in the tiller bed nursery were evaluated agronomically during 1951 and 1952 in two experiments in an adjacent field. Experiment I consisted of duplicate polycross and single topcross entries for each of 19 clones and 141 of the possible 171 singlecrosses among the same clones. One clone was dropped because of insufficient seed. Experiment II included 12 of the 20 clones with one bulk topcross and one polycross entry each and eight separate entries per clone each representing a different replication from the topcross nursery. Objectives were to determine possible differences among clones in combining ability and the extent to which polycross or topcross progeny performance resembled mean singlecross performance. Of interest also, was the consistency in performance both between and within years under broadcast planting conditions.

Agronomic performance

Abbreviated analyses of variance for the two experiments are given in Table 4 and 5. F tests for the various progeny types were made using appropriate error components. Methods of separation for the entry x replication error mean square are illustrated in Table 5. Because little would be gained thereby, years x entries and years x entries x replications mean squares were not separated on a progeny type basis.

In Experiment I highly significant mean differences were found among singlecrosses for all characteristics both years and in the combined analyses. Polycrosses differed significantly for disease score and bloom date in 1951, panicle number in 1952, and panicle number in the two-year analyses. Differences among topcrosses resembled those for polycrosses, except that disease score differences were not significant. In all tests for significance, however, F values for polycrosses were higher than those for topcrosses. Mean differences among progeny types all were significant, except for bloom date. Singlecrosses averaged higher in spring vigor, panicle number, and forage yield and were later in blooming than polycrosses or topcrosses. Grand means for panicle number were 51.1, 39.3, and 43.5 panicles per four square feet for singlecrosses, polycrosses, and topcrosses, respectively. Forage yield means were 4.86, 4.83, and 4.65 pounds per plot in the same order. These results suggest as expected, a greater expression of heterosis by the singlecrosses. Disease reactions were not the same from reading to reading

Table 4. Analyses of variance of green forage yields and other agronomic characteristics in orchardgrass progeny tests in 1951 and 1952. Experiment I.

Source of variation	D.F.	Mean squares				
		Spring vigor score 1951	Date of blooming 1951	Disease score 1951	Panicle No. 1951-52	Green forage yield 1951-52
Replications	2	43.98	109.59	33.19	257.69	66.34
Types of progeny	2	4.52*	9.08	4.37**	13,842.90**	2.27*
Singlecrosses	140	2.09**	16.58**	3.35**	2,432.09**	1.15**
Topcrosses	18	1.72	20.94**	0.82	1,323.33**	0.68
Polycrosses	18	2.59	24.75**	2.25**	2,104.69**	1.07
Between duplicate polycrosses	19	1.29	3.44	0.77	320.74	0.73
Replications x entries	394	1.16	5.32	0.53	585.38	0.52
Years[1/]	1	----	----	219.80*	474,960.03	166.72
Years x replications	2	----	----	3.29	110,923.01	14.41
Years x entries	197	----	----	0.55**	1,093.45**	0.40**
Years x entries x replications	394	----	----	0.33	569.57	0.28

*$P < 0.05$; **$P < 0.01$

[1/] Substitute readings for years in disease score analysis

Table 5. Analyses of variance of panicle numbers and forage yields in Experiment II for 1951-1952 combined.

Source of variation	D.F.	Mean squares: Panicle number	Mean squares: Forage yield
Replications	2	1,828.50	47.35
Among clones[1]	11	9,016.36**	2.59**
Within clones[1]	84	967.37**	0.65
Bulk topcrosses	11	1,105.36**	1.44
Polycrosses	11	1,953.00**	1.16*
Types of progeny	2	2,030.50	0.40
Error	238	461.61	0.71
Replications x among clones	22	439.23	0.62
Replications x within clones	168	501.15	0.69
Replications x bulk topcrosses	22	259.64	1.22
Replications x polycrosses	22	372.73	0.50
Replications x types of progeny	4	523.75	0.20
Years	1	598,522.00**	155.02**
Replications x years	2	3,993.50	1.55
Year x entries	119	773.19**	0.39
Replications x years x entries	238	411.00	0.32

[1] Each of 12 clones represented by one seed entry from each of eight replications in topcross nursery.

* $P < 0.05$; ** $P < 0.01$

nor did entries differ to the same degree in panicle number or forage yield from year to year. Average panicle number and forage yield for all entries, however, were similar in 1951 and 1952.

In Experiment II highly significant differences in mean panicle number were obtained for each type of progeny. Differences in mean forage yield among polycrosses and among clones (mean of replicated topcrosses) were significant at the 5 per cent and 1 per cent levels, respectively. Mean yearly effects also differed significantly for both characteristics, while differences among entries in panicle number were not the same from year to year.

Results of progeny tests in both experiments suggest that the clones studied differed in combining ability for several agronomic characteristics. Bulk topcross performance appeared least efficient for detecting such differences. Several possible reasons may be offered. First, the nature of the topcross nursery was such that somewhere between 25 and 50 per cent of the germ-plasm represented by each topcross progeny came from the common-pollinator parent. This common parentage may have had a masking effect on expression of general combining ability differences and a consequent decrease in range of performance. Singlecross and polycross progenies would not be affected in this manner. A smaller range for bulk topcrosses than for singlecrosses or polycrosses was at least one factor in lower F values. Less experimental accuracy may have been another explanation. However, in neither experiment was there a consistent tendency for bulk topcrosses x replications mean squares to be higher than those for polycrosses, singlecrosses, or replicated topcrosses. Polycross means in Experiment I and replicated topcross means in Experiment II were based on 6 and 24 plots, respectively, whereas bulk topcross means were based on only three plots in both experiments. This could indicate a more efficient determination of means in the former instances. Irrespective of the reasons for lack of significant differences among bulk topcross entries for several traits, however, it appears that three replications of a randomized block design would not be very effective for evaluating combining ability by use of a topcross progeny test in orchardgrass.

Interannual and intraseasonal correlations

Forage yield and panicle number were the only characteristics studied in both 1951 and 1952 in the progeny tests. Interannual correlations for these traits for each type of progeny in Experiment I appear in Table 6. All but one were significant statistically, though none was of high predictive value. As noted in the clonal nursery, forage yields were somewhat more consistent than panicle numbers for the two years. This substantiates the higher F value for the years x entries mean square for panicle number in the combined analyses (Table 4). Of particular interest were the correlations involving mean singlecross performance of each clone. In these cases from 33 to 54 plots (depending on the number of singlecrosses involving a clone) were used to calculate mean yield or panicle number for a clone. Relative differences among even these mean values appeared to vary considerably from year to year, thus further indicating the need for at least two years' data for progeny evaluation. It also should be pointed out that none of the interannual correlations in

Table 6. Interannual correlations for green forage yields and panicle numbers of singlecross, topcross, and polycross progenies of 19 orchardgrass clones at Ames, Iowa in 1951 and 1952. Experiment I.

Character	Individual singlecrosses (139 d.f.)	Mean of singlecrosses (17 d.f.)[1]	Top-crosses (17 d.f.)	Poly-crosses (17 d.f.)
Green forage yield	0.43**	0.56*	0.53*	0.58**
Panicle number	0.38**	0.42	0.49*	0.51*

[1]Averages of all singlecrosses involving each clone used for correlations.
*P < 0.05 ; **P < 0.01

the progeny tests was as high as those found in the parental nursery for the same two years and traits. These results agree with those previously reported by Weiss, et al. (28).

Intraseasonal correlations between first and second cutting yields were positive and highly significant for singlecross and polycross progenies but not for topcrosses in 1951, but all were low and nonsignificant in 1952 (Table 7). Here again the degree of association was less than observed in the tiller bed nursery in the same two years. Correlations between disease readings, however, did not follow the same pattern. June and August readings were positive and significantly associated in the progeny tests, while little or no association was noted in the clonal nursery. Apparently the broadcast plantings provided a more favorable environment for maintenance of a similar disease complex than the more open tiller bed nursery. It is worthy of mention that intraseasonal correlations were less for topcross than for singlecross or polycross progenies in 1951, which lends further support to previous statements of less efficiency for topcrosses in the evaluation of combining ability.

Table 7. Intraseasonal correlations for green forage yields and disease susceptibility scores of singlecross, topcross, and polycross progenies of 19 orchardgrass clones at Ames, Iowa in 1951 and 1952. Experiment I.

Item correlated	d.f.	1951	1952
First and second cutting yields:			
Singlecrosses	139	0.61**	0.21
Topcrosses	17	0.36	0.30
Polycrosses	17	0.60**	0.14
Disease susceptibility scores:			
Singlecrosses	139	0.69**	- -
Topcrosses	17	0.46*	- -
Polycrosses	17	0.59**	- -

*P < 0.05 ; **P < 0.01

Table 8. Intercharacter correlations for singlecross, polycross, and topcross progenies of 19 clones in Experiment I in 1951 and 1952.

Item correlated	Year	Singlecrosses (139 d.f.)	Polycrosses (17 d.f.)	Topcrosses (17 d.f.)
Disease score and:				
Panicle number	1951	-0.50**	-0.07	-0.29
Forage yield	1951	-0.26**	0.18	0.04
Spring vigor score and:				
Panicle number	1951	0.51**	0.46*	0.67**
Forage yield	1951	0.68**	0.73**	0.42
Bloom date and:				
Panicle number	1951	-0.31**	-0.13	-0.21
Forage yield	1951	0.13	0.23	0.08
Panicle number and:				
Forage yield	1951	0.44**	0.32	0.28
	1952	0.29**	-0.03	0.40
	1951-52	0.21	-0.02	0.39

*P < 0.05; **P < 0.01

Intercharacter correlations

Correlations among characteristics evaluated in 1951 and 1952 in Experiment I are presented in Table 8. Early growth vigor was significantly associated in a positive manner with panicle number and forage yield of all progeny types, except for forage yield of topcross progenies. Significant negative correlations were obtained between disease score and both panicle number and forage yield and between bloom date and panicle number for singlecrosses but they were of rather low predictive value. Correlations between panicle number and forage yield of singlecross progenies were positive and highly significant in 1951 and 1952 but were not significant for the two years combined. These intercharacter correlations for the three types of progenies present a somewhat different picture than those among clones, and suggest that selection for high combining ability for a combination of desirable traits may not be as difficult as indicated by clonal performance.

Parent-progeny and Interprogeny Relationships

Because of the close similarity in environmental conditions under which the clonal and progeny tests were conducted, it was possible with correlations to compare efficiency of clonal, singlecross, polycross and topcross progenies for evaluation of combining ability. Consequently, all possible parent-progeny and interprogeny correlations for Experiments I and II were calculated. These are given in Tables 9, 10, and 11. Single year as well as two-year progeny and three-year parental means were

Table 9. Parent-progeny and interprogeny correlations for spring vigor and disease susceptibility scores and date of blooming in 1951. Experiment I

	Topcrosses	Polycrosses	Singlecrosses
Parental clones			
Spring vigor score	0.24	0.50*	0.50*
Disease score	0.42	0.28	0.49*
Date of blooming	0.51	0.60**	0.59**
Topcross			
Spring vigor score	- -	0.33	0.47*
Disease score	- -	0.57*	0.69**
Date of blooming	- -	0.67**	0.61**
Polycross			
Spring vigor score	- -	- -	0.40
Disease score	- -	- -	0.86**
Date of blooming	- -	- -	0.72**

*P < 0.05 for 17 d.f. **P < 0.01 for 17 d.f.

used for panicle number and forage yield, while only 1951 data were used for spring vigor and disease scores and for bloom date. In all cases mean singlecross performance of a clone was considered the best estimate of combining ability for each particular characteristic.

Disease scores and bloom dates of topcross and polycross progenies were highly correlated with mean singlecross performance, although polycross progenies were more similar in rank (Table 9). Clonal performance for the same traits also was significantly associated with singlecross response, but at a somewhat lower level. Three of the six parent-progeny and interprogeny correlations for spring vigor were significantly positive (5 per cent level) but were considered of rather low predictive value.

In Experiment I performance of polycrosses and topcrosses for panicle number likewise was similar to singlecross performance, especially in 1952 and for 1951-52 combined (Table 10). Parent-progeny associations for the same trait were of some predictive value but to a lesser degree. Mean topcross performance in Experiment II (each clone mean based on 24 plots) was predicted fairly well by both clonal and polycross progeny results, though the latter were more closely related on a two-year basis. Bulk topcrosses apparently performed in a more dissimilar manner than other types of progeny in the second experiment, since none of the correlations involving them were statistically significant.

Data in Table 11 show that only forage yields of polycross progenies had an appreciable relationship with singlecross yields in Experiment I. Parental and polycross yields in Experiment I also were significantly correlated (5 per cent level), although no apparent reason for such an association is evident. Most other parent-progeny and interprogeny correlations for yield were positive but low in value.

Table 10. Parent-progeny and interprogeny correlations for panicle numbers in 1951 and 1952 and for 1951-52 combined in Experiments I and II.

Item		Topcrosses Expt. I Bulk	Topcrosses Expt. II Bulk	Topcrosses Expt. II Mean	Polycrosses Expt. I	Polycrosses Expt. II	Single Crosses Expt. I
Parental clones[1]	1951	0.23	0.56	0.83**	0.31	0.61*	0.55*
	1952	0.50*	0.21	0.61*	0.40	0.48	0.47*
	1951-52	0.39	0.38	0.69*	0.48*	0.57	0.53*
Topcrosses (bulk)	1951	- -	- -	0.39	0.12	0.34	0.32
	1952	- -	- -	0.56	0.49*	0.36	0.78**
	1951-52	- -	- -	0.54	0.52*	0.52	0.70**
Polycrosses	1951	- -	- -	0.72**	- -	- -	0.51*
	1952	- -	- -	0.80**	- -	- -	0.75**
	1951-52	- -	- -	0.89**	- -	- -	0.81**

[1]Three-year averages (1950-52) for parental clones used in correlations with 1951-52 progeny performance.
*$P < 0.05$ for 17 d.f. in Experiment I and 10 d.f. in Experiment II.
**$P < 0.01$ for 17 d.f. in Experiment I and 10 d.f. in Experiment II.

Table 11. Parent-progeny and interprogeny correlations for annual green foliage yields in 1951 and 1952 and 1951-52 combined in Experiments I and II.

Item		Topcrosses Expt. I Bulk	Topcrosses Expt. II Bulk	Topcrosses Expt. II Mean	Polycrosses Expt. I	Polycrosses Expt. II	Single Crosses Expt. I
Parental clones[1]	1951	0.16	0.11	0.18	0.53*	0.40	0.34
	1952	0.07	0.02	0.39	0.51*	-0.06	0.27
	1951-52	0.02	0.07	0.28	0.51*	0.20	0.26
Topcrosses (bulk)	1951	- -	- -	0.50	0.36	-0.03	0.17
	1952	- -	- -	0.30	0.42	0.06	0.33
	1951-52	- -	- -	0.46	0.42	-0.11	0.22
Polycrosses	1951	- -	- -	0.14	- -	- -	0.64**
	1952	- -	- -	-0.22	- -	- -	0.61**
	1951-52	- -	- -	-0.12	- -	- -	0.65**

[1]Three-year averages (1950-52 for parental clones used in correlations with 1951-52 mean progeny performance.
*$P < 0.05$; **$P < 0.01$ for 17 d.f. in Experiment I

Table 12. Correlations of agronomic performance of outcross progenies of 12 orchardgrass clones in Experiment I and in Experiment II based on 1951-52 means.

Experiment I	Experiment II					
	Polycross		Mean Topcross		Bulk Topcross	
	Yield	Panicle no.	Yield	Panicle no.	Yield	Panicle no.
Mean single cross:						
Yield	-0.02		0.86**		0.23	
Panicle no.		0.64*		0.80**		0.62*
Polycross:						
Yield	0.06		0.80**		0.49	
Panicle no.		0.56		0.70*		0.82**
Topcross:						
Yield	0.22		0.38		0.22	
Panicle no.		0.80**		0.77**		0.62*

*P < 0.05; **P < 0.01 for 10 d.f.

As a final comparison of the different progeny types, results for yield and panicle number in Experiment I were correlated with those obtained in Experiment II. These correlations appear in Table 12. All but one intertest correlation for panicle number was significant at the 5 per cent or 1 per cent level. In the case of forage yield, however, only mean singlecross and polycross progeny performance in the first experiment were highly associated with mean topcross performance in the second experiment. All other intertest correlations for yield were low and non-significant.

These parent-progeny, interprogeny, and intertest correlations suggest several pertinent conclusions regarding evaluation of combining ability in orchardgrass. First, it appears that singlecross, topcross, and polycross progenies evaluate differences among clones in general combining ability in a similar manner. Secondly, results indicated that the degree of association in performance among progeny types was influenced considerably by the accuracy of mean estimates of progeny performance. This was particularly true for forage yield. Mean singlecross and mean topcross values were most accurately estimated and showed the highest correlations. Polycross progenies in Experiment I had duplicate entries and also were strongly associated in relative performance with mean topcross and singlecross progenies. Bulk topcross means in both experiments and polycross means in Experiment II were based on only single entries of three replicates and tended to be lower in predictive value, especially for yield. Parental clone performance was of value in predicting combining ability to some degree, but generally was less efficient than polycross or topcross performance. A final analysis might be that it appears possible to use either polycross or topcross progenies

for evaluating combining ability in orchardgrass if progeny tests are sufficiently replicated to give reasonably accurate estimates of mean performance for important characteristics. Use of replicated clonal tiller bed nurseries for this purpose hardly seems justified, not only because of greater cost and time involved and lesser efficiency but also because in the final analysis outcross progeny tests still would need to be grown.

DISCUSSION

Weiss, et al. (28) suggested several possible reasons for the low associations for forage yield obtained among clonal, singlecross, and open-pollination progenies in orchardgrass. These were use of wide-spaced row plantings rather than tiller bed or broadcast plots, inadequate estimates of mean performance due to low number of replications, lack of genetic variability, and effects of nonrandom pollination in production of open-pollinated seed. Present investigations involved clonal material common to both studies and the use of clonal tiller bed and broadcast planting methods. Thus, results obtained herein can be related to their data and suggestions. Lack of genetic variability did not appear to be a valid explanation, since in this subsequent study differences in clonal performance and combining ability were evident for all characteristics. Inadequate replication, however, seemed to be a primary cause of low associations in outcross progeny performance, since magnitude of correlation coefficients tended to increase with greater accuracy in determining the means. This was especially true for forage yield.

Despite differences in planting methods, clones performed more consistently than seedling progenies in both studies. Associations in clonal performance between the two planting methods were low for yield and disease readings and high only for date of bloom, indicating that the tiller bed planting ranked clones differently for forage yield potential. In contrast with wide-row spaced planting, use of the tiller bed nursery for clonal evaluation increased the year to year consistency in performance to some extent, but appeared to have little effect on the degree of association between parents and outcross progenies for yield or any other characteristic. Use of broadcast rather than 3-foot drill row plantings for progeny tests, likewise, resulted in some increased consistency in character expression from year to year and within years. There also was an indication that the degree of relationship in performance between different progeny types was improved by use of solid plantings.

Considering these results, it seems that lack of adequate replication is the most important factor causing low associations in yield performance among progeny types. Planting method is important probably because of planting method x entry interactions rather than because of failure of any one planting method to differentiate entries if replication is sufficient. Consequently, use of broadcast or solid drilled plots for evaluation of combining ability in orchardgrass appears most logical not only in light of results reported by Weiss and Mukerji (27) but also because they probably would show more consistency in performance than wide-row or spaced plantings. Failure of clonal nursery performance to predict combining ability for forage yield apparently cannot be explained by lack of replication or planting effects. The real reason appears to be a low heritability.

As previously stated, use of singlecrosses for evaluating combining ability in orchardgrass is hardly feasible. Presently, many forage breeders feel that the polycross progeny test is the first and best alternative for this purpose. Theoretically, such reasoning is justified, since with random equal cross-pollination in the nursery and precise evaluation in the progeny test, polycross and mean singlecross performance of a group of clones should be identical if all possible singlecrosses are made and tested and all clones occur in the polycross nursery. Several studies have been shown recently, however, that the assumption of random, equal cross-pollination in polycross nurseries is not justified (7, 10, 30). It is to be expected that as numbers of selections in the nursery increase and as the range in date of blooming becomes greater, the chances of approaching random intercrossing become less and less. In addition, the cost and mechanics of establishing polycross nurseries with many replications in the hope of attaining random mating would seem almost prohibitive if more than 25 to 50 plant selections were included. From the standpoint of the progeny test itself, polycross progenies have no special advantage, since they give only a measure of general combining ability if numbers are large and means are accurately estimated.

In light of these disadvantages and the fact that topcrosses appeared as efficient in evaluating differences in combining ability in the present investigations when means were estimated accurately, it seems appropriate to suggest several reasons favoring greater utilization of topcross testing in grass breeding. In the first place, only sufficient replications are needed in the nursery to provide enough seed for testing. Four to eight single plant replicates usually are adequate for this purpose. Contrasted with the much larger number of replications needed in a polycross nursery, many more selections can be included in a topcross nursery with the same number of plots. Since large numbers of selections usually must be tested to find the few which are inherently superior, greater advances may be permitted by topcross testing. In addition, the mechanics of planting, plot design, seed harvesting, and seed bulking are simpler with fewer replications. A second advantage of topcross testing is that differences among selections in bloom date in a topcross nursery are of lesser importance, since an adapted variety used as the common-pollinator in alternate rows can be expected to show a range in genetic composition for maturity. This leads to a third reason. The effects of nonrandom mating on efficiency of evaluation for combining ability would probably be much less in a topcross than in a polycross nursery. A final advantage is that single plants can be spaced rather far apart in a topcross nursery to insure crossing with the common pollinator. Such spacing would facilitate note taking, if desired. In a polycross nursery, on the other hand, close and equal spacings in all directions are necessary to aid in the attainment of random and equal crossing.

On the basis of results and discussion presented herein, a tentative breeding plan for orchardgrass might be proposed as follows:

Year	Procedures
1	Establish space-planted nursery of promising material from local and introduced sources. (Replicated rows or plots of different sources would facilitate separation into generally desirable or undesirable entries for selection purposes). Evaluate single plants for vigor of establishment.
2	Select plants for desired maturity and leaf type, winterhardiness, general vigor and leafiness, and disease resistance. Clip several times to note recovery. Transfer selected plants to greenhouse in fall for propagation.
3	Establish maintenance nursery of a few propagules of each clone which are not allowed to set seed, thereby assuring continued purity of stand. Establish topcross nursery of several replications using spaced, single plant replicates and randomized complete block design. Plant alternate rows to adapted commercial strain as a common pollinator. Include extra rows of common pollinator on all sides of nursery (Fig. 1). Notes on clones on a replicated basis can be taken for vigor, leafiness, disease resistance, etc.
4	Harvest and thresh seed separately for each plant. Save a few panicles per plant to determine open-pollinated fertility. Eliminate low-fertility clones and others producing few panicles or exhibiting other undesirable features such as winter susceptibility or low spring vigor on a replicated basis. Bulk seed on equal weight basis from each replication of a clone to make up topcross entry. Seedling vigor and disease resistance tests could be conducted in fall or winter and poor performers discarded.
5, 6, 7	Establish topcross test of survivors in solid-planted plots with minimum of 5 or 6 replications at one or several locations as feasible. Evaluate agronomically for at least two years, especially for forage production, recovery, disease resistance, spring vigor, and perhaps leafiness. Include commercial strains as checks.
8	Establish isolated recombination block for production of ultimate synthetic variety or for recurrent selection purposes using clonal material from maintenance nursery. Include only the highest combiners, as determined by topcross test, in recombination.

This outline could be modified to suit the needs and facilities of the individual breeder. A comparable plan already has been initiated at the Iowa Station with bromegrass and is approximately halfway through the cycle.

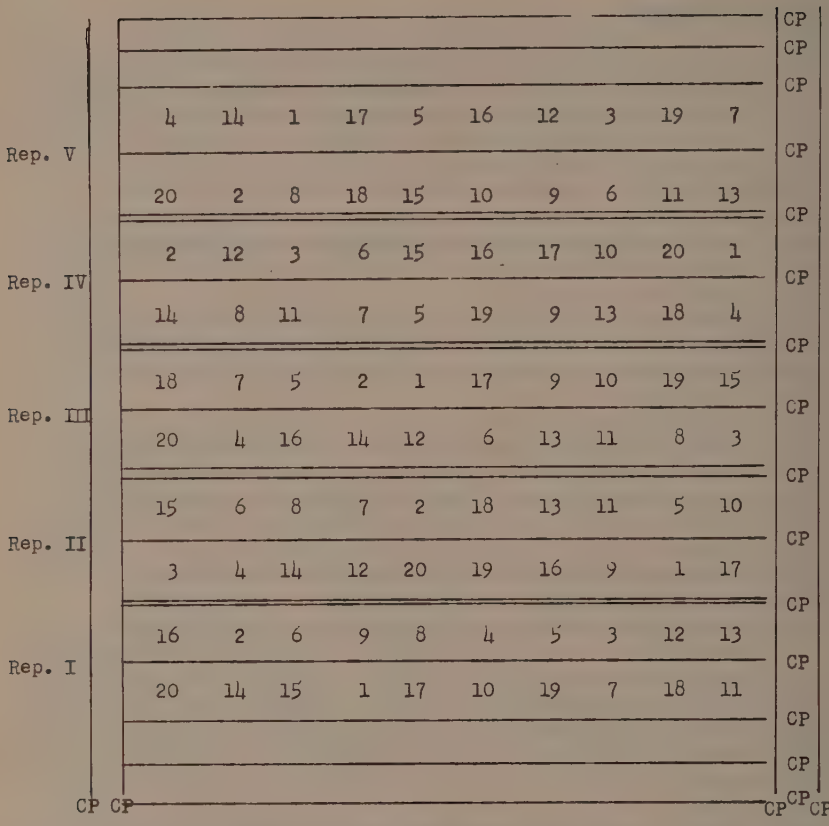

Fig. 1. Proposed plan of topcross nursery of 20 clones of orchardgrass using rows spaced 3 feet apart with plant spacing of 4 feet within rows and five replications of a randomized block design. (Rows at right angles to prevailing wind direction). This plan can be extended to include about 400 clones in an acre field. CP designates common pollinator row. The number of border rows may be modified to meet specific needs for isolation.

The primary difference between this and many other breeding outlines is the elimination of the replicated clonal nursery as such and substitution of a topcross for the polycross test for evaluation of combining ability. Characteristics exhibiting relatively high heritability, such as bloom date, disease resistance, winterhardiness, leaf width, and perhaps even panicle number, spring growth capacity, fertility, and leafiness can be selected for in the topcross nursery on a replicated basis (15, 28). Clones considerably below average in expression of desired combinations of these characteristics could then be eliminated before topcross testing with a minimum of lost time and effort. Rigorous selection for agronomically

desirable, late blooming clones would be necessary as results indicated a general negative relationship between bloom date and certain other characteristics in the clonal nursery and progeny tests. Selection for rapid spring growth, leafiness, good aftermath recovery capacity, and winterhardiness would tend to increase forage production, while clones high in fertility and good in panicle production should be good seed producers.

A final point worthy of mention in regard to the suggested program relates to utilization of legume companion crops. Overseeding of the original space-planted nursery or of the topcross nursery with ladino clover, trefoil, or alfalfa after the plants are well established would certainly be feasible and desirable. Likewise, legume mixtures also could be used in the topcross test although it might be best to wait until synthetic varieties are available for testing with legumes.

Several other phases relating to the problem of evaluating combining ability in orchardgrass have been carried out concurrently with this investigation. One study pertains to the number of replications desired in a polycross or topcross nursery. Another is an attempt to estimate the relative importance of general and specific combining ability, as expressed by the material at hand, and the effects of varying number of testers. A third explores the relationships among self- and cross-fertility, clonal performance, and combining ability. Results of these studies will be presented in subsequent publications. In addition, synthetic varieties of five high and five low combining clones are being produced, and will be tested to determine if previous estimates of potential yield were essentially correct.

SUMMARY

1. A group of orchardgrass clones selected from long-time stands in Iowa and their singlecross, polycross, and topcross progenies were evaluated agronomically to study interrelationships between clonal performance and combining ability. Clones were evaluated from 1950 to 1953 in a replicated tiller bed nursery, while outcross progenies were tested in 1951 and 1952 in two broadcast experiments in an adjacent field.

2. Results indicated that the clones differed genetically for a number of agronomic traits and in combining ability for disease resistance, bloom date, spring growth vigor, panicle production, and forage yield. On the assumption that mean singlecross performance gave the best estimates of combinind ability of a clone, polycross and topcross progenies each generally predicted combining ability in a similar manner when mean estimates were reasonably accurate. Considering all traits, clonal performance was of least value in predicting combining ability, though parent-singlecross correlations for date of blooming, spring vigor and disease scores, and panicle number were statistically significant. Low parent-progeny associations for forage yield appeared to be due primarily to low heritability and not to effects of planting method or lack of genetic variability.

3. Interannual correlations and entry x year interactions in the analyses of variance were not always in agreement, although both provided information relating to time necessary for evaluation of tests. Parental clones were more consistent from year to year and within years than outcross progenies for all characteristics except leaf disease ratings. All data suggest the need for evaluation several times a year for disease susceptibility and forage yield and continuing for at least two years for these and other desirable characteristics.

4. In the clonal nursery associations among spring vigor, leafiness, panicle number, and forage yield generally were positive and correlations statistically significant. Bloom date showed a negative relationship with forage yield, panicle number, and spring vigor. Height, leaf disease intensity, and seed yield were essentially unrelated to other characteristics. Intercharacter correlations in the progeny tests were of lesser magnitude although significant positive associations between spring vigor and panicle number and between spring vigor and forage yield were found. Late blooming and disease susceptible progenies tended to produce fewer panicles, but none of these traits was of much predictive value for yield.

5. Discussion of advantages and disadvantages of using polycross and topcross tests for evaluating combining ability indicated that the latter appear to merit more consideration for use in orchardgrass breeding. A proposed outline of a breeding program using the topcross test was presented.

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FUNGI FROM BIRDS ASSOCIATED WITH WILTED OAKS IN IOWA[1]

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The problem of the dissemination of the oak wilt fungus (Endoconidiophora fagacearum Bretz), is one of the primary questions confronting the investigators in this field. Recently Norris (1) and Dorsey, Jewell, Leach, and True (2) have demonstrated that insects of the family Nitidulidae can transmit the fungus. During the time these experiments were being made, the possibility of transmission by birds, particularly woodpeckers, was also under investigation, since in the case of chestnut blight Heald and Studhalter (3) showed that birds were carriers of Endothia parasitica (Murr.) And., the fungus causing chestnut blight. Although Endoconidiophora fagacearum Bretz was not recovered from the birds investigated, the fungous flora associated with them proved sufficiently interesting to warrant a report.

Birds were trapped at intervals during the growing season in the diseased areas of Pilot Knob and Call State Parks with particular attention being paid to the woodpeckers that were visiting the oaks in these areas. The beaks and throats of the trapped birds were swabbed with sterile cotton and the swabs streaked on sterile media in petri dishes. The resulting colonies of fungi were isolated and identified. One 1953 collection was composed of fungi isolated from birds that had been shot after they had been observed on or near wilted trees on which fungal mats were being produced.

Four hundred and forty colonies of fungi were isolated from three hundred and six birds. In the 1952 isolation, the woodpeckers, downy and red-bellied, carried the greater number of fungi while the hairy woodpeckers, chickadees, and nuthatches carried fewer (Table II).

The 442 isolations fell into 41 genera (Table I). In 1952, they were most frequent in the May 9th collection (3.2 per bird) having increased from 1.1 per bird taken on March 8th. Collections made later in May fell to approximately one per bird and remained fairly constant throughout the remainder of the summer.

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[1]Journal Paper No. J-2438 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1047. In cooperation with the National Oak Wilt Research Committee and the Iowa State Conservation Commission.

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TABLE I

The distribution of fungi by genera from birds collected in oak wilt areas in Iowa in 1952 and 1953

Kind of fungus	Times isolated		Kind of fungus	Times isolated	
FUNGI IMPERFECTI					
Moniliaceae			Sphaerioidaceae		
Candida	1		Phoma	19	
Geotrichum	4		Cytospora	10	
Aspergillus	16		Coniothyrium	31	
Penicillium	52		Pyrenochaeta	2	
Paecilomyces	4		Ascochyta	3	
Scopulariopsis	2		Phomopsis	3	
Cephalosporium	7		Sphaeropsis	2	
Trichoderma	75		Diplodia	2	
Sporotrichum	8		Total		72
Sporotrichella	2				
Hyalopus	1		PHYCOMYCETES		
Verticillium	2		Mucorales		
Pachybasium	2		Mucor	13	
Total		176	Rhizopus	2	
Dematiaceae			Mortierella	1	
Pullularia	14		Thamnidium	1	
Papularia	1		Absidia	1	
Nigrospora	2		Total		18
Cladosporium	30				
Alternaria	72		ASCOMYCETES		
Helminthosporium	1		Sphaeriales		
Total		120	Otthia	1	
Melanconiaceae			Didymella	1	
Pestalozzia	1		Thielavia	1	
Total		1	Chaetomium	9	
Stilbaceae			Melanospora	1	
Graphium	11		Total		13
Total		11			
Tuberculariaceae			BASIDIOMYCETES		7
Fusarium	13				
Epicoccum	11		Grand Total		442
Total		24			

Identification of species within the genera was made where possible. However, in many of the genera of the Fungi Imperfecti the identity of the species has been dependent on the host or substrate upon which the fungus was growing in nature so that in these genera no specific identification could be made since origin of the spores on the birds beaks was unknown. In these cases the cultures were described without specific names. A similar situation is to be found in the ascomycetous genera. In order that others may have a basis for recognizing as clearly as possible just what fungi were found, descriptions and illustrations have been included.

TABLE II

Number of fungi collected on birds in oak wilt areas in 1952 and 1953

Kind of bird	Number of birds 1952	Number of birds 1953	Number of fungi 1952	Number of fungi 1953
Downy woodpecker	188	16	293	15
Hairy woodpecker	74	6	52	12
Chickadee	26	--	33	--
Nuthatch	13	3	4	6
Red-bellied woodpecker	2	--	3	--
Blue Jay	2	--	2	--
Redhead	--	1	--	7
Brown creeper	--	3	--	19

Key to the genera

a. Mycelium nonseptate; asexual spores in sporangia; sexual spores the result of fusion of like gametangia.
  b. Sporangia all of one kind
    c. Sporangia columellate
      d. Sporangia pyriform . . . . . Absidia
      dd. Sporangia globose
        e. Sporangia clustered on nodes opposite rhizoidal-like branchlets . . . . . Rhizopus
        ee. Sporangia scattered, not at nodes . . . . . Mucor
    cc. Sporangia without columellae Mortierella
  bb. Sporangia of two kinds, 1) large with columellae and 2) sporangioles without columellae, with few spores. Thamnidium
aa. Mycelium septate; asexual spores on conidiophores; sexual spores either ascospores or basidiospores
  b. Ascospores present, in ascomata
    c. Ascomata without ostioles, asci indeterminately scattered within the ascomata; ascospores dark, one-celled . . . . . Thielavia
    cc. Ascomata ostiolate
      d. Ascomata superficial; ascospores one-celled
        e. Perithecia dark, covered with long variously ornamented hairs . . . . . Chaetomium
        ee. Perithecia bright-colored, with long neck. Melanospora
      dd. Ascomata sunken, without long hairs; ascospores two-celled.
        e. Ascospores hyaline . . . . . Didymella
        ee. Ascospores colored . . . . . Otthia
  bb. Conidia only present, neither ascospores nor basidiospores present.
    c. Conidia in pycnidia
      d. Conidia hyaline
        e. Conidia all of the same kind

f. Pycnidia monoloculate
g. Conidia one-celled
h. Pycnidia smooth . . . . . Phoma
hh. Pycnidia with setae about the ostiole . . . . . Pyrenochaeta
gg. Conidia two-celled . . . . . Ascochyta
ff. Pycnidia pluriloculate . . . . . Cytospora
ee. Conidia of two kinds, 1) globose to elongate and 2) thread-like . . . . . Phomopsis
dd. Conidia dark
e. Conidia one-celled
f. Conidiophores obsolete, conidia small. Coniothyrium
ff. Conidiophores long filiform, conidia large Sphaeropsis
ee. Conidia two-celled . . . . . Diplodia
cc. Conidia not in pycnidia
d. Conidia in acervuli . . . . . Pestalozzia
dd. Conidia not in acervuli
e. Conidia scattered, on separate conidiophores;
f. Conidia and conidiophores both hyaline or bright-colored
g. Conidia one-celled globose, ovate or short cylindric
h. Conidiophores short, or obsolete or little different from vegetative hyphae
i. Conidia in unbranched chains. . Geotrichum
ii. Conidia in branching chains . . . Candida
hh. Conidiophores long and distinct from the vegetative hyphae.
i. Branching of conidiophores confined to the tip, conidia in heads.
j. Conidia not in chains
k. Conidiophores simple
l. Conidia in mucoid heads Hyalopus
ll. Conidia not in mucoid heads . . . Cephalosporium
kk. Conidiophores branched Trichoderma
jj. Conidia in chains
k. Conidiophores swollen at tip Aspergillus
kk. Conidiophores verticillately branched at the tip
l. Colonies green; phialides short. . . . Penicillium
ll. Colonies not green, phialides long and tapering
m. Conidia globose to elliptic without basal ring Paecilomyces
mm. Conidia truncate at base with basal ring Scopulariopsis
ii. Branching of conidiophores not confined to the tip
j. Branching of conidiophores not in whorls

k. Conidia globose to ellipsoid Sporotrichum
kk. Conidia fusoid to cylindric Sporotrichella
jj. Branching of conidiophores in whorls
k. Apical branchlets of conidiophores sterile, conidia on lower branches on short flask-shaped phialides . . . . . Pachybasium
kk. All branchlets bearing spores . . . . . Verticillium
ff. Conidia and conidiophores one or both dark-colored
g. Conidiophores not differentiated from the mycelium
h. Conidia one-celled
i. Conidia budding from dark septate mycelial threads . . . . . Pullularia
ii. Conidia single on short side branches Papularia
gg. Conidiophores differentiated from the mycelium
h. Conidia not in chains, single on inflated tip of conidiophores . . . . . Nigrospora
hh. Conidia in chains
i. Conidia two-celled, elongate, on branching conidiophores . . Cladosporium
ii. Conidia more than two-celled
j. Conidia many-celled by cross-walls . . . . . Helminthosporium
jj. Conidia muriformly divided. . Alternaria
ee. Conidiophores gathered into coremia or sporodochia
f. Conidiophores in coremia . . . . . Graphium
ff. Conidiophores in sporodochia
g. Conidia fusiform, curved, bright-colored. . Fusarium
gg. Conidia dark, globose, muriformly divided . . . . . Epicoccum

Order MUCORALES

Family MUCORACEAE

1. Absidia van Tieghem

Mycelium found as in Rhizopus with the sporangiophores grouped in clusters of two to five on the incurved arches between the nodes (point of rhizoidal origin); sporangia erect, equal, pyriform, furnished with an infundibuliform apophysis, sporangial wall diffluent leaving a short basal collarette; columellae hemispheric, conic or mammiform, more rarely spinescent or terminated by a single long prolongation, with a cross-wall a definite distance below the sporangium; spores small, 5-6 microns, round or oval (not angular) with a smooth wall, rarely echinulate, colorless or bluish-black; zygospores formed on the stolons, surrounded by circinate appendages in a whorl from one or both suspensors, gametangia straight.

Absidia coerulea Bainier (Pl. I, a)

Filaments of the thallus bluish-violet, continuous, unequally branched, at times knotted, sporangiophores single, borne directly on the thallus, attaining 25 mm. in length, ended by an infundibuliform apophysis; septa 12-24 microns from the tip; sporangia uniform, globular, 36-42 microns, changing from pale violet to gray, then to brown, membrane smooth, diffluent, leaving a collarette; columellae hemispheric or obconic, often ending in a papilla; spores numerous, small, smooth, pale violet, globose, 4-7 microns; zygospores not seen.

2. Rhizopus Ehrenberg

Mycelium of two kinds, one submerged in the substratum and the other aerial, constituting the arching filaments (stolon); stolons arch from point to point on the substrate producing rhizoids at the points of contact (nodes) with the sporangiophores arising from the nodes, singly or in groups of two to five; sporangiophores erect, with their tips enlarged into an apophysis with the columella inserted above the point where the spherical bend attaches to the filament, white at first becoming bluish-black at maturity, globose to sub-globose, flattened at the base; wall not cuticularized, uniformly incrusted and entirely diffluent leaving no collarette; columellae broadly subjacent, hemispherical, forming after dehiscence, by collapse, an orgen of the shape of the pileus of a mushroom; spores round or oval, angular, colorless or colored, bluish or brown, smooth or striate, rarely spinulose; zygospores naked, formed in the substrate and on the stolons; suspensors straight, very large and swollen, without appendages.

Rhizopus nigricans Ehrenberg (Pl. I, b)

Stolons creeping, recurving to the substrate in the form of arachnoid hyphae, which are strongly-raised and distant from the substrate and implanted at each node by means of rhizoids; internodes often attain a length of 1-3 cm. and the hyphae are more or less branched; sporangiophores rarely single, united in groups of three to five or more, 0.5-4 mm. in height x 24-42 microns in diameter; apophysis broad, cuneiform; sporangia hemispheric, 100-350 microns; columellae broad, hemispheric, depressed, 70 microns in diameter by 90 microns high (250 x 320 microns maximum); spores unequal, irregular round or oval, angular striate, 9-12 x 7.5-8 microns, gray-blue.

3. Mucor Micheli

Mycelium widespread in and on the substratum, but without rhizoids on special membered stolons, richly branched with tapering hyphae, straight or knotted, at first continuous, in age with irregularly distributed septa and smooth colorless walls; sporangiophores springing singly from the mycelium but usually forming a thick turf, erect, either unbranched or branched with like sporangia on all branch ends; branching in part monopodial, clustered or irregularly panicled or umbelliferous, in part cymose and more or less sympodial, curved, with sporangia at the tip of the sympodium, never dichotomous; sporangia erect at all times, a few

weakly bent, usually all alike, only of different size, many spored, globose, opening while still on the sporangiophore, only a few of the sympodial forms abscissing while still closed, of various colors; sporangial walls not cuticularized, incrusted more or less strongly with needles of calcium oxalate, dissolving quickly in water leaving a collarette or breaking and then at times persistent; columellae always present, of various shapes, colorless or colored; spores spherical or ellipsoid with thin smooth wall, colorless or colored; zygospores on the mycelium, naked, suspensors without appendages; gametangia straight.

Mucor varians Povah (Pl. I, c)

Turf 1-3.5 cm tall on bread, ivory yellow to olive-buff; sporangiospores 8-20 microns in diameter, either little or profusely branched, much coiled, twisted or intertwined, forming a dense tough cottony turf with proliferations of hyphae and columellae often present. Sporangia globose or sub-globose, smooth, 60-80 microns in diameter, at first yellow or pale orange, then very dark gray, tinged with green at maturity; wall diffluent leaving a basal collarette; columellae free or slightly adnate, very variable in shape, sub-globose, hemispherical, flattened hemispherical, oval, cylindrical, elliptical, pyriform, panduriform, subconical; large columellae hemispherical to conical, small columellae cylindrical to pyriform, 25-50 x 20-45 microns; membrane tinged gray with or without orange contents; spores various, oval to subelliptical, 4-6 x 3-4 microns.

Mucor fragilis Bainier

Turf gray to brown, of various heights, 2-15 mm; sporangiophores erect about 6-15 microns in diameter, usually with marked sympodial branching, helicoid; sporangia, when moist, yellowish-white to gray, later olive-brown; when dry, beige to gray brown, later olive-brown; small, 35-96 microns, wall more or less slowly fragmenting; columellae globose to oval, smooth, hyaline, up to 50 microns high, with a more or less marked collarette; spores in mass dark-brown, with regular elliptic-cylindrical shape, twice as long as wide: 2-4 x 4-8 microns; mycelial gemmae detached.

Mucor mucedo (L.) Bref.

Sporangiophores erect, forming a very raised turf up to 15 cm in height, silvery-gray, shining, not branched, 2-15 cm high x 30-40 microns in diameter, without crosswalls; wall colorless, smooth; content colorless tardily yellow; sporangia large, 100-200 microns in diameter, at first yellow then deep gray or brownish-black; membrane of sporangium very diffluent, leaving a collarette, encrusted with needle-shaped crystals of calcium oxalate; columellae free, cylindric or campanulate or spherical, 70-140 microns long x 50-80 microns wide, with colorless wall and red orange content; spores elliptic or subcylindric, twice as long as broad, of various sizes in the same sporangium, 6-12 microns long x 3-6 microns wide with a smooth hyaline wall, content tardily yellow or colorless.

Mucor hiemalis Wehmer (Pl. I, d, e)

Sporangiophores usually unbranched, erect, then prostrate by wilting; turf about 1 cm high, close and fine, cottony, white, rarely grayish-yellow; sporangia spherical, gray or brownish-yellow, visible to the naked eye, 52 microns in diameter; wall diffluent, leaving a collarette; columellae free, spherical or oval, colorless, 28-48 microns (spherical) or 25 x 21 to 36 x 29 microns; spores usually unequal, the majority elongate, ellipsoid or kidney-shaped, 7 x 3.2 microns (3-8.4 x 2-5.6 microns), smooth hyaline with a thin membrane.

Family MORTIERELLACEAE

4. Mortierella Coemans

Mycelium very fine and delicate; nutritive mycelium submerged, much branched at times forming cysts; aerial mycelium prostrate, many times anastomosing, sporangiophores erect, short at first, becoming rapidly elongate, thread-like with limited growth, simple or branched, very broad below, diminishing to the tip; sporangia terminal, globose, without columellae; membranes then diffluent, spores globose or ellipsoid; zygospores globose covered by a thick case; conidia formed on short side branches on the aerial mycelium, spherical, one-celled.

Mortierella turficola Ling-Young (Pl. I, f)

Turf snow-white, woolly, composed of orbicular tufts of sinuous or lobed hyphae; hyphae creeping, hyaline, 2.5-10 microns in diameter, forked, filled with dense, finely granular protoplasm; sporangiophores 4-6 microns wide, larger at the base, diminishing at the tip, 120-170 microns high, ending in a solitary sporangium; sporangia globose, smooth, hyaline, 24-29 microns in diameter, with hyaline membrane; spores globose, smooth, hyaline, 1.8-2 microns in diameter, without trace of nucleus or vacuoles.

Family THAMNIDIACEAE

5. Thamnidium Link

Sporangiophores erect, terminated by a sporangium resembling that of the genus Mucor; the sporangia are formed at definite points on single or verticillate branches that in turn are many times dichotomously branched and terminated by small sporangioles; terminal sporangia multisporous, with diffluent membrane, incrusted with calcium oxalate crystals and with a large columella; sporangioles small, spherical, containing four to ten spores, with incrusted, persistent membrane, without columellae, caducous; spores of the same size in both sporangia and sporangioles, colorless, smooth; zygospores naked, on the mycelium; suspensors without appendages; gametangia straight.

Thamnidium elegans Link (Pl. I, g)

Turf 3 cm high; sporangiophore bearing a terminal sporangium, 100-200 microns in diameter, with a columella 50-70 microns wide x 62-90

microns long; lateral branches divided in whorls of dichotomous branchlets; the length of the branch is diminished in proportion to its forking; the first arm from the place of insertion on the principal filament to the first fork, 150-200 microns long; the arm of the first order, 40–60 microns, the last are 4-6 microns long x 2 microns in diameter; sporangioles, very variable in size, up to 24 microns in diameter; the smaller have no more than four, often only two or one spore; spores always the same size in all sporangia, 8-12 x 6-8 microns.

Family ASPERGILLACEAE

6. Thielavia Zopf

Cleistothecia globose; walls brown, pseudoparenchymatic without ostioles, and without appendages; asci oval, eight-spored; ascospores irregularly arranged in the ascus, brown, one-celled.

Thielavia terricola (Gilman and Abbott) Emmons (Pl. II, a, b, d)

Colonies on corn meal agar broadly spreading, composed of white, cottony aerial hyphae and submerged hyphae, 1-6 microns in diameter, branches constricted at base, homothallic; ascocarps arising from an ascogonial coil, spherical, without ostiole, 80-125 microns in diameter, reaching 250 microns in older cultures, brownish to black at maturity; asci oval to pyriform, 16-19 x 25-35 microns, deliquescing within the cleistothecium; ascospores broadly fusiform, slightly apiculate at both ends, dark olivaceous to brown, 7-9 x 10-16 microns, with wall much thickened at ends opposite the germ pore.

Family CHAETOMIACEAE

7. Chaetomium Kunze

Perithecia superficial, subglobose or elongate, dark and more or less opaque, ostiolate; wall membranaceous, brittle provided with appendages in the form of variously modified hairs; asci thin-walled, delicate, stalked evanescent, club shaped or cylindrical, eight-spored; ascospores one-celled, colored, typically lemon-shaped.

Chaetomium dolichotrichum Ames (Pl. II, c, e)

Culture at first white to gray becoming smoky olive with perithecial formation; perithecia olive to smoky-gray, globose to subglobose, 100-150 microns in diameter, produced early in center of culture; lateral hairs few, short, brown becoming lighter at tip, 3-5 microns wide at base, tapering to a blunt round point, smooth to slightly roughened, regularly septate; terminal primary hairs extremely long, unbranched, smooth or only slightly roughened, dark brown, regularly septate 5.5 microns at base; secondary terminal hairs forming a compact mass, dichotomously branched, light brown, septate, minutely roughened, varying from 2.5-3 microns wide; ascospores light brown, oval or ovoid 6 x 4-5.5 microns.

Family SPHAERIACEAE

8. Didymella Sacc.
(Pl. III, c, e)

Perithecia sunken, erumpent by a papillate mouth, globose; walls thin, black, smooth; asci cylindrical to club-shaped; with a double wall, 8-spored; ascospores ellipsoid or oval, two-celled, hyaline; paraphyses present. A single species with spores 30.6 x 6.8 microns. Perithecia produced after several months on agar slants. Vegetative mycelium gray, abundant, no conidia observed.

9. Otthia Nitschke
(Pl. III, f)

Perithecia gregarious, sunken and then erumpent, globose or ovoid, black; walls thick, leathery, smooth; ostioles small, wart-like; asci cylindric, 8-spored; ascospores ellipsoid, two-celled, brown; paraphyses present, A single species with spores 10-12 x 3-4 microns. Perithecia produced near dried edges of agar slants. Vegetative mycelium greenish gray, abundant, no conidia observed.

Family NECTRIACEAE

10. Melanospora Corda
(Pl. III, a, b, d)

Perithecia superficial, without stroma, globose-pyriform, with a long neck, usually clothed at the tip with a fringe of hairs and perithecia often hairy; asci broad-clavate, 4-8 spored; spores simple, colored, brown or brownish-black. A single species with spores 17-22 by 9-10 microns. Young perithecia with very short neck or a simple tuft of long hairs. Perithecia produced profusely on agar in two weeks. Vegetative mycelium white, no conidia observed.

Family SPHAERIOIDACEAE

11. Phoma (Fr.) Desmazieres
(Pl. IV, a)

Pycnidia at first covered, then erumpent, membranous to leathery or almost carbonaceous, globose or slightly lens-shaped, smooth, with a small apical papilla; conidia small, oval spindle-form, cylindric or almost globose, one-celled, hyaline, often biguttulate; conidiophores thread-like, seldom short or almost lacking, simple.

Two species were encountered, one with conidia 5-7 x 3-4 microns, the other with conidia 2-4 x 1-2 microns. Isolates varied somewhat in cultural characteristics. In some, little aerial mycelium was formed, and the culture consisted of a mass of pycnidia that produced spores in such abundance that they covered the culture in a pink, slimy mass. In others, abundant vegetative mycelium was produced and the pycnidia were produced in it. Culture types were not associated with spore size.

12. Pyrenochaeta de Not.

Pycnidia spherical, flask-shaped, single and erumpent, membranous or almost carbonaceous, black, with stiff simple setae chiefly at the top, ostiolate. Conidia oval, elongate to cylindrical, almost hyaline, one-celled, conidiophores rod-like, branched. Sparse vegetative mycelium produced; pycnidia were formed at the agar surface, and became more erumpent as they approached maturity.

13. Ascochyta Libert
(Pl. IV, b)

Pycnidia membranous, globose to lens-shaped, with an ostiole; conidia oval or elongate, two-celled, hyaline or light green.

A single species with conidia 9-12 x 3-5 microns. Abundant gray aerial mycelium was formed, often obscuring the pycnidia. Pycnidia superficial, spores often exuding in a mass.

14. Cytospora Ehrenberg
(Pl. IV, d)

Pycnidia in covered stromata, becoming erumpent, globose to flask-shaped divided into irregular or radially arranged locules; conidia allantoid, one-celled, hyaline, erumpent in cirrhi; conidiophores various. A single species with pycnidia 900-810 x 750 microns and conidia allantoid, 4-6 x 1-2 microns. These cultures were readily recognized by their curious gray to brown tufted mycelium which was rather appressed. Intense brown coloration developed in the agar in a short time. The multiloculate pycnidia were formed in about fourteen days, commonly first around the inoculum or at the edge of the agar. Cream colored cirrhi developed profusely in some isolates.

15. Phomopsis Sacc.

Pycnidia lens-, bolster-shaped, or globose, sclerotial with an internal hyaline tissue, and external black wall with variously formed ostioles; conidia long, egg-shaped or usually spindle-shaped with 2 oil-drops, conidiophores thread-like usually longer than the spores; in addition to the primary conidia a second type of thread-like curved or S-shaped conidia occur.

Abundant vegetative mycelium was formed, the pycnidia developing near the surface of the agar. Both spore types were present in abundance.

16. Coniothyrium Corda
(Pl. IV, c, e, f)

Pycnidia submerged and then erumpent or almost superficial, globose or flattened, with papillate ostiole, black, membranous to carbonaceous; conidia globose or ellipsoid, small, dark, one-celled; conidiophores short, simple or almost lacking.

The species are separated by hosts; hence those encountered in this

study are practically impossible to ascribe to a given name. Three species are found to have the following spore sizes.

I. Conidia elongate 3-4 x 2 microns
II. Conidia elongate 5-10 x 2-4 microns
III. Conidia globose 3-4 microns

As was true of the Phoma isolates, several cultural types were observed here. Production of vegetative mycelium varied, but pycnidia were always quite readily produced. In some isolates, spores were produced in such abundance that they gave the colony a slimy appearance.

17. Sphaeropsis Lev.

Pycnidia covered, erumpent, globose with a papillate ostiole, thick-walled, of parenchymatic, externally black, internally hyaline tissue; conidia oval or elongate, brown, one-celled; conidiophores rod-like, hyaline.

Pycnidia were produced sparsely on the various media used. Spores were 12 to 15 microns in diameter.

18. Diplodia Fries
(Pl. IV, g)

Pycnidia submerged and then erumpent, carbonaceous, black, usually with a papillate ostiole; conidia ellipsoid or oval, two-celled, dark; conidiophores rod-like, simple, hyaline.

A single species with pycnidia 375-450 microns in diameter and ellipsoid conidia, dark, 10-13 x 3-4 microns. Not appreciably different in gross appearance from isolates of Ascochyta or Phomopsis.

Family MELANCONIACEAE

19. Pestalozzia de Not.

Hymenium submerged, finally erumpent, shield- or cushion-form, dark; conidia elongate, three- to many-celled, colored, sometimes with the end cells hyaline on the tips with one or more hyaline hairs; conidiophores thread-like.

Pestalozzia torulosa B. and C. (Pl. IV, h)

Conidia 5-celled, clavate, torulose or strongly constricted at the septa usually curved, 20.4-24.3 microns; colored cells olivaceous, 17.5 x 5.2-7 microns, end cells hyaline, the apical cell cylindric, the basal cell conic tapering to a short pedicel; setae 2-3, divergent, 10.5-14 microns. Vegetative growth was somewhat restricted, spores produced abundantly.

Family MONILIACEAE

20. Geotrichum Link
(Pl. V, a)

Hyphae prostrate, septate, forming a turf; conidiophores short, erect or ascending, septate, breaking up into conidia in chains at their apices;

conidia short, cylindric, truncate at both ends, or slightly rounded, hyaline or pale.

21. Candida Berkout

Hyphae scanty, prostrate, breaking up into segments of various length; conidia produced by budding on the hyphae or at their tips, small, one-celled, hyaline, in branching chains.

22. Cephalosporium Corda

Sterile hyphae creeping; conidiophores arise as short lateral branches of aerial hyphae, erect, nonseptate, not swollen at the tip; conidia borne singly at the tips of conidiophores, being pushed aside as they are formed successively, usually ovate, hyaline or slightly colored. Isolates were quite different in general appearance and in abundance of spores produced.

23. Hyalopus Corda
(Pl. V, c)

Sterile hyphae prostrate, sparse; conidiophores erect, usually nonseptate, hyaline, not or slightly swollen at their tips; conidia hyaline or bright-colored, enclosed in a mucus to form a head.

Vegetative hyphae somewhat appressed, conidial heads gave colony a somewhat zonate appearance.

24. Trichoderma (Persoon) Harz

Sterile hyphae prostrate, septate, forming a flat, firm turf; conidiophores erect, arising from short, branched side-branches; branching usually opposite, not swollen at the apex and bearing terminally the conidial heads; conidia small, mostly globose, bright-colored or hyaline.

Trichoderma album Preuss

On bean agar, colonies thin, spreading, small; white tufts of aerial mycelium and conidiophores arise from aerial mycelium, branched, up to 25 or 30 microns in length, bearing terminal heads up to 15 microns in diameter; conidia elliptical to oval, hyaline, 2.5-3.2 x 1.5-2 microns.

Trichoderma lignorum (Tode) Harz (Pl. V, b)

Colonies on Czapek's agar broadly spreading, hyaline, fruiting areas appear as tufts, white at first, and becoming various deep green shades with age; reverse colorless; conidiophores arise as branches of aerial mycelium, septate, up to 70 microns in height x 3 microns in diameter, di- or trichotomously branched, occasionally forming whorls; conidial heads up to 10 microns in diameter; conidia globose to ovate, smooth, 3.8-3.2 microns.

25. Aspergillus (Micheli) Corda

Vegetative mycelium consisting of septate branching hyphae, colorless; conidial apparatus developed as stalks and heads from specialized, enlarged, thick-walled hyphal cells (foot-cells) producing conidiophores

as branches approximately perpendicular to the long axis of the foot-cells; conidiophores nonseptate or septate, usually enlarging upward and broadening into elliptical, hemispherical or globose fertile vesicles bearing phialides, either parallel and clustered in terminal groups or radiating from entire surface; phialides in one series, or as a primary series, each bearing a cluster of two to several secondary phialides at the apex; conidia varying greatly in color, size, shape, and markings, successively cut off from the tips of the phialides by cross-walls and forming unbranched chains arranged into radiate heads or packed into columnar masses; cleistothecia found in certain species only; sclerotia regularly found in some strains, occasionally found in others.

Key to the species of Aspergillus

a. Conidial heads some shade of green
  b. Conidiophores with smooth walls
    c. Vesicle cylindrical clavate, conidiophores coarse. A. clavatus
    cc. Vesicle flask-shaped or globose, not cylindrical clavate
      d. Phialides in one series
        e. Conidia globose. . . . . A. fumigatus
        ee. Conidial elliptic to pyriform . . . . A. restrictus
      dd. Phialides in two series
        e. Conidial chains in columns; cleistothecia present . . . . . A. nidulans
        ee. Conidial chains in radiate heads
          f. Heads blue-green . . . . . A. sydowi
          ff. Heads glaucous-green or yellow-green A. versicolor
  bb. Conidiophores with pitted or rough walls; heads yellow-green . . . . . A. flavus
aa. Conidial heads never green
  b. Conidiophore walls smooth
    c. Conidial heads brown
      d. Conidial heads olive-brown . . . . . A. ustus
      dd. Conidial heads dark brown to black . . . . . A. niger
    cc. Conidial heads orange to umber . . . . . A. wentii
  bb. Conidiophore walls pitted or rough, heads ochraceous . . . . . A. quercinus

Aspergillus clavatus Desmazieres (Pl. V, e)

Colonies upon Czapek's agar growing rapidly at 20-24°C, plane or slightly furrowed, in certain strains tending to become floccose but generally characterized by a surface mycelial mat and abundant erect conidiophores up to 3 mm in length, bearing large, blue-green, clavate conidial heads evenly distributed or arranged in more or less well defined zones; reverse generally uncolored, but becoming browned in age in some strains; odor strongly foetid in some strains, not pronounced in others; conidial heads clavate, large, commonly ranging from 300 to 400 microns by 150 to 300 microns, in age splitting in 2, 3 or more divergent columns of compacted conidial chains, approximately slate-olive in color; conidiophores 1.5-3 mm in length, 20-30 microns in diameter, comparatively thin-walled, smooth, colorless, gradually enlarging at the apex into a

clavate vesicle which is fertile over an area up to 200-250 microns in length and 40 to 60 microns or more wide; phialides in a single series, varying in size from 2.5-3.5 x 2-3 microns at the base of the vesicle to 7-8 or occasionally 10 x 2.5-3 microns at its apex; conidia elliptical, comparatively heavy walled, smooth, 3-4 x 2-3 microns, occasionally larger in some strains and irregular in others.

Aspergillus fumigatus Fresenius (Pl. V, f)

Colonies upon Czapek's agar spreading broadly over the substratum, in some strains strictly velvety, in others more or less floccose with varying amounts of tufted aerial mycelium to deep felted or extremely floccose forms, white at first, becoming green with the development of heads but varying considerably in the final shade of green, often becoming dark green or almost black in age; reverse and substratum in some strains uncolored, in others showing varying amounts of yellow, or again passing over in dark red shades in age; conidial heads columnar, compact, varying in measurement from strain to strain up to 400 x 50 microns, but usually much shorter, occasionally very small; conidiophores short, smooth, usually densely crowded, up to 300 microns (in occasional strains up to 500 microns) in length by 2-8 microns in diameter, frequently more or less green colored, especially in the upper part, arising directly from submerged hyphae or as very short branches from aerial hyphae, septate or unseptate, gradually enlarging upward and passing almost imperceptibly into the apical flask-shaped vesicles; vesicles up to 20-30 microns in diameter, usually fertile on the upper half only; phialides in one series, usually about 6-8 (varying from 5-10 x 2-3 microns, crowded, closely packed with axes roughly parallel to the axis of the conidiophore; conidia dark green in mass, echinulate, globose, mostly 2.5-3 microns in diameter with extremes ranging from 2-3.5 microns; sclerotia or perithecia not found. Commonly present in compost and other materials undergoing decomposition at high temperatures.

Aspergillus restrictus G. Smith

Colonies growing very poorly on Czapek's agar; growing moderately well on wort agar, dark dull green, gradually turning gray or brownish-gray; reverse in some cultures uncolored, in others green to dark green; surface velvety at first, becoming wrinkled and often acquiring a warted appearance; heads forming long, compact, slender columns up to 350 x 20-30 microns; conidiophores arising mostly from substratum but also as branches of aerial hyphae, commonly 50-100 microns, occasionally 150-200 x 3-3.5 microns, often with one or two septa, smooth, sinuous, uncolored; vesicles flask-shaped 7.5-14 microns in diameter; phialides in one series, borne on upper surface of vesicles only, 6-9 x 2.5-3 microns; conidia rough, spinulose, elliptical or somewhat pyriform, often showing a distinct connective, dark greenish-brown, 4-6.5 x 3-4 microns, mostly 4.5-6 x 3-3.5 microns; perithecia not found. Young conidia are hyaline and cylindrical and appear to be segments of enormously elongated septate phialides. They gradually swell without increasing in length, at the same time becoming pigmented, but even in old heads they adhere strongly together in columns of parallel chains.

Aspergillus nidulans (Eidam) Wint. (Pl. VII, a, b, c)

Colonies upon Czapek's agar plane, spreading broadly, dark cress green from abundant conidial heads during the first two weeks; perithecia developing from the center of the colony outward after the first few days, separately produced, often abundant; sectoring occasional; reverse of colony in varying shades of purplish-red during the growing period, becoming very dark in age; heads short, columnar, ranging from 40-80 x 25-40 microns, commonly 50-70 x 30-35 microns; conidiophores commonly sinuous, with walls smooth, in shades of cinnamon brown, ranging from 60-130 microns, commonly 75-100 microns in length, about 2.5-3 microns near the foot increasing to 3.5-5 microns below the hemispherical vesicle; vesicle 8-10 microns in diameter; phialides in two series, primary 5-6 x 2-3 microns and secondary 5-6 x 2-2.5 microns; conidia globose, rugulose, 3-3.5 microns in diameter, green in mass.

Perithecia developed separately within or upon the conidial layer, globose, ranging from 100-175 microns in diameter, commonly 125-150 microns with outer layer a yellowish to cinnamon colored envelope of scattered hyphae bearing "hulle " cells up to 25 microns in diameter; wall composed of one layer of cells, dark reddish-purple; in ripening becoming a mass of 8-spored asci which break down quickly leaving the ascospores free; ascospores purple-red, lenticular, smooth-walled with 2 equatorial crests, spore bodies about 3.8-4.5 x 3.5-4 microns, equatorial crests pleated with margin sinuous and entire, ranging from 0.5-1 micron in width.

Aspergillus sydowi (Bainier and Sart.) Thom and Church (Pl. VI, b)

Colonies on Czapek's agar growing well at room temperature, in most strains close-textured and velvety from crowded conidiophores and heads arising from the substratum, in other strains more or less floccose from interlacing and trailing aerial hyphae bearing conidial heads ranging from large, well-formed structures to minute fruits consisting of clusters of simple phialides bearing few conidial chains, blue-green in color, approximating Delft blue with the blue effect especially marked in young fruiting areas; reverse usually in shades of red, from coral red to maroon to almost black in some strains in age; conidial heads typically radiate to nearly globose, ranging from 100-150 microns, but often reduced to small penicillate clusters of phialides especially in marginal colony areas and upon aerial hyphae; conidiophores mostly arising from submerged hyphae, up to 500 x 5-8 microns, colorless, smooth, comparatively thick-walled; vesicles nearly globose, fertile over almost the entire surface, up to 20 microns in diameter; phialides in two series; primary 6-7 x 2-3 microns, secondary 7-10 x 2-2.5 microns; conidia globose 2.5-3 microns, in our culture up to 3.5 in diameter, conspicuously spinulose, green en masse; globose (hulle) cells closely resembling those of the A. nidulans group have been seen in occasional strains. No sclerotia or perithecia are found, but clamydospores in solid substrata are reported.

Aspergillus versicolor (Vuill.) Tiraboschi (Pl. V, d)

Colonies upon Czapek's agar rather slow growing, compact, in some strains velvety and consisting almost entirely of closely crowded conidio-

phores arising from the substratum, in other strains showing a marked development of floccose hyphae bearing more or less abundant conidiophores as short aerial branches, in still others a combination of both growth types with colony centers initially floccose and outer areas almost velvety, at first white, passing through shades of yellow, orange-yellow, tan to yellowish-green shades such as pea green or sage green depending upon the strain and conditions of culture, occasionally with the green colors almost or completely lacking; reverse and substratum occasionally colorless or nearly so, mostly passing through shades of yellow to orange, then rose, purple-red or red, the particular shade and intensity of color normally persisting as a strain or varietal character; heads roughly hemispherical, radiate, up to 100 to 125 microns in diameter; rarely approaching columnar; conidiophores colorless, smooth, up to 500 or even 700 x 5 microns or approaching 10 microns near the vesicles; vesicles 12-20 microns in diameter, fertile area hemispherical or semi-elliptical, passing almost imperceptibly into the funnel-like enlarged apex of the conidiophore ; phialides in two series, primary commonly 8-10 x 3 microns, occasionally less, secondary 5-10 x 2-2.5 microns; conidia globose, usually delicately echinulate, mostly 2.5-3 microns, occasionally 3.5-4 microns, usually borne in loosely radiating chains.

"Hulle" cells of the A. nidulans type are occasionally seen. Neither perithecia nor sclerotia have been found.

Aspergillus flavus Link (Pl. VI, a)

Colonies on Czapek's agar spreading rapidly, with floccosity limited to scanty growth of sterile hyphae in older and dryer areas among crowded conidiophores; conidial areas range in color in various strains from sea-foam yellow through chartreuse yellow, citron green, lime green to even ivy green, yellow green colors are either persistent or, in old colonies, altered by the disappearance of the green factor leaving shades of yellow-brown; reverse yellowish at first, passing over into brown shades in age; conidial heads vary from small with a few chains of conidia to large radiate or columnar masses in the same culture and varying mixtures of different types and sizes of head; conidiophores mostly arising from submerged hyphae, commonly 400-100 x 5-15 microns, with walls pitted, rough, almost spiny in appearance, broadening upward and gradually enlarging into vesicles 10 to 30 or 40 microns in diameter, dome-like in the smaller heads, flask-shaped in larger heads; phialides in a single series in many smaller heads, or both single and double series on the same vesicles in large heads, varying from single phialides only 10-15 x 3-5 microns to primary phialides 7-10 x 3-4 microns and a secondary series 7-10 x 2.5-3 microns; conidia pyriform to almost globose, nearly colorless to definitely yellowish green varying from 3, 3 x 4, 4 x 5 microns, or even larger and marked variously with pits, echinulations, or irregularly winding color bars and ridges to give a roughened effect of varying intensity. Sclerotia, when found, at first white then brown, hard parenchymatous, and a few strains white tipped, produced by some strains regularly and abundantly, scantily by others under undefined conditions. Perithecia not found.

Aspergillus ustus (Bainier) Thom and Church (Pl. VI, c)

Colonies upon Czapek's agar spreading broadly, plane, sulcate or umbonate, rarely zonate, more or less felted or floccose; at first white, becoming olive-gray, yellow-brown, fuscous or russet to purplish vinaceous with the development of mature conidial structures; generally heavy sporing, with some conidiophores arising from the substratum but more abundantly from aerial hyphae; reverse in shades of yellow, orange, and brown to almost black in age; odor not pronounced; heads radiate to irregularly hemispherical, sometimes loosely columnar, commonly splitting into more or less well-defined columns in age, very variable in size, ranging in color from dull green or olive-gray, through grayish-brown to fuscous or fuligineus; conidiophores arising from submerged hyphae, ranging up to 500 x 3-6 microns, aerially borne conidiophores, ranging from very short up to 125 x 2-5 microns, sinuous, sparsely septate, with walls rather thin, smooth, and uniformly colored some shade of brown; vesicles hemispherical to subglobose, 8-20 microns in diameter, smaller in some strains; phialides colorless or colored, semi-radiate, loosely arranged into two series, primary phialides 4-7 x 3 microns, secondary phialides 5-7 microns x 2-2.5 microns; conidia globose, 3.5-5 microns, roughened, echinulate to marked with conspicuous color bars, ranging from greenish through olive-gray to yellow-brown to fuligineus. Many strains producing thick-walled "hulle" cells ranging in form from irregularly ovate or elongate in some strains, to serpentine, helicoid, or twisted in others, essentially as in Aspergillus flavipes.

Very common in soil and decaying vegetation.

Aspergillus niger van Tieghem (Pl. VI, e)

Colonies rapidly growing with abundant submerged mycelium, colorless, or in some strains with more or less yellow color in the hyphae and in the substratum, with aerial hyphae usually scantily produced, but abundant in age in certain strains; conidial heads fuscous, blackish-brown, purple-brown, in every shade to carbonaceous black, typically globose or radiate, commonly up to 300, 500 or occasionally 1000 microns in diameter with periphery variously splitting into radiating columns of conidia; small heads, more or less columnar and consisting of a few conidial chains often borne on trailing hyphae or short conidiophores near the substratum; conidiophores mostly rising directly from the substratum, uncolored or yellow to brown near the vesicle only, smooth with walls thick, frequently uneven on the inner surface and splitting lengthwise into strips when broken, nonseptate or with occasional thin septa, varying greatly in length and diameter in different strains and in colonies of different media or even in sections of the same colony, thus ranging from strains with conidiophores 200-400 x 7-10 microns to forms with conidiophores several millimeters long and 20 microns or more in diameter; vesicles globose to subglobose, thick-walled, commonly 20-50 microns, occasionally up to 100 microns in diameter, colorless or more commonly more or less intensely yellow-brown; phialides in one series in young colonies and in small heads, but typically in two series, colorless at times, usually more or less intensely brown, even carbonaceous, primary phialides closely packed, covering the vesicle, varying greatly in size in the same colony but usually 20-30 microns by 6-8 microns;

secondary phialides more uniform ranging usually from 6-10 x 2-3 microns, both series often more or less brown to almost black; conidia globose when ripe, with walls at first smooth with diffused brown or fuscous color, then rough or spinulose from coloring substance deposited as tubercles, bars or loops between the outer primary wall and inner, or secondary wall, mostly 2.5-4 microns, occasionally up to 5 microns in diameter. Sclerotia globose, superficial, regularly produced by certain strains, sporadically by some, and not found in many others.

Aspergillus wentii Wehmer

Colonies on Czapek's agar rapidly growing and broadly spreading, floccose with white or yellowish aerial hyphae which in some strains pile up in the plate or fill the test tubes for several centimeters, but remain inconspicuous in other strains, with developing heads at first white through yellow shades to olive-brown, medal bronze or snuff brown, or coffee brown to chocolate brown, reverse becoming reddish-brown in old cultures; conidial heads large, globose, generally remaining radiate in age, ranging up to 500 microns in diameter, changing from yellow shades to brown; conidiophores up to several millimeters in height by 10-25 microns in diameter, with walls colorless up to 4 microns in thickness, studded with droplets in growing colonies and often appearing slightly roughened when examined dry, but uniformly smooth in fluid mounts; vesicles globose or nearly so, varying up to 80 microns in diameter, fertile over the entire surface; phialides usually in two series, primaries 10-20 x 3-5 microns, occasionally larger, secondaries 6-8 x 3 microns; conidia borne in long chains, more or less elliptical, ranging from 3.5-6 microns in long axis, but mostly 4-5 microns, double wall clearly evident, ranging from almost smooth to marked by ridges sometimes suggestive of A. niger, again more closely resembling the A. flavus series. No perithecia reported. Sclerotia often encountered, dark brown to black, ovate with long axis vertical.

Aspergillus quercinus (Bainier) Thom and Church (Pl. VI, d)

Colonies upon Czapek's agar spreading broadly, characterized by the presence of an aerial white mycelium and the abundant production of sclerotia over the whole area, or in sectors, or variously distributed; the mass changing from yellow to orange-yellow and finally to rufous or brick red shades with the ripening of the sclerotia; reverse in shades of yellowish- range; conidial heads in yellow tints, near sulfureus, scattered among the sclerotia or occurring in long-stalked groups in the dryer areas of the culture tube or plate, mostly up to 200 microns in diameter, but occasionally larger, up to 300 or even 400 microns; conidiophores with walls mostly pale yellow, especially in the outer layer, pitted, occasionally with abundant granules, about 2 microns in thickness, varying from short and inconspicuous in crowded sclerotial areas to very long tufts in dryer areas, up to 2 or even several millimeters by 10 to 20 microns; vesicles colorless, crushing easily, 35-45 microns in diameter, fertile over the entire surface; phialides in two series; primary 10-20 or even 30 microns in larger heads by 2-4 or 7 microns at the tips; secondary 10 x 2-2.5 microns; conidia 2.5-3 x 3-3.5 microns to almost globose, very nearly colorless in mounts, smooth, thin-walled. Sclerotia white to brick red, up to 500 microns in diameter.

26. Penicillium Link

Vegetative hyphae prostrate, septate, branched; conidiophores erect, usually unbranched, septate, at the apex with a verticil of erect primary branches, each with a verticil of secondary (metulae) and sometimes tertiary branchlets or with a verticil of conidia-bearing cells (phialides) borne directly on the slightly inflated apex of the conidiophores, sometimes with secondary conidiophores borne on the apex of the main conidiophore; conidia borne in chains which typically form a brush-like head, not enclosed in slime; well-differentiated foot-cells not present; conidia globose, ovate or elliptical, smooth or rough.

Key to the species of Penicillium

a. Penicilli typically in single verticils of phialides borne on branches which maintain the identity of each verticil
  b. Conidiophores generally unbranched
    c. Colonies velvety or nearly so, with conidiophores arising mostly from the substratum . . . . . P. frequentans
    cc. Colonies loose-textured, with conidiophores as short branches from interlacing hyphae . . . P. decumbens
  bb. Conidiophores mostly branched
    c. Conidia globose, in divergent chains . . . . P. waksmani
    cc. Conidia ovate to slightly elliptical, in parallel chains forming columns . . . . . P. charlesii
aa. Penicilli characteristically once- or twice-branched below the level of the phialides.
  b. Penicilli typically asymmetrical, phialides not lanceolate
    c. Colonies typically velvety
      d. Penicilli strongly divaricate; conidial chains strongly divergent; phialides abruptly tapered . . . P. janthinellum
      dd. Penicilli seldom strongly divaricate; conidial chains tending to become columnate
        e. Colonies bright yellow to orange-pink in reverse; conidial areas in bright blue-green shades . P. citrinum
        ee. Colonies dull yellow to olive-buff in reverse; conidial areas dull blue-green or yellow-green shades . . . . . P. steckii
    cc. Colonies typically lanose or floccose
      d. Conidiophores commonly long, usually arising from aerial hyphae, not in bundles
        e. Conidia globose or nearly so, less than 4 microns in diameter, finely roughened. . . . . P. lanosum
        ee. Conidia elliptical, 4 microns or more in diameter, smooth
          f. Conidial areas blue-green to gray-green . . . . . P. lanoso-coeruleum
          ff. Conidial areas at first court gray to gnaphalium green, becoming olive. . P. commune
      dd. Conidiophores aggregated into fascicles or coremiform bundles

e. Colonies with simple conidiophores and fascicles intermixed, chiefly simple
f. Colonies typically yellow-green in conidial areas . . . . . P. palitans
ff. Colonies typically blue-green
g. Colonies in dull blue-green shades mostly azonate; conidiophores roughened
h. Colonies granular and tufted . . P. cyclopium
hh. Colonies with fasciculation reduced, velvety . . . . . P. puberulum
gg. Colonies brighter blue-green shades, narrowly zonate; conidiophores smooth
h. Colonies spreading, heavily sporing on malt agar . . . . . P. martensii
hh. Colonies more restricted, non-sporing on malt agar . . . . P. aurantio-virens
fff. Colonies typically in pale to dull gray-green shades . . . . . P. urticae
ee. Colonies with most of the conidiophores arranged in fascicles
f. Conidia globose to subglobose, yellow-green shades . . . . . P. corymbiforme
ff. Conidia elliptical, pale blue-green shades . . . . . P. granulatum
bb. Penicilli biverticillate and symmetrical
c. Colonies with conidiophores arising primarily from aerial hyphae or ropes of hyphae . . . P. funiculosum
cc. Colonies with ropiness absent
d. Colonies with deep red colors in reverse
e. Conidia rough . . . . . P. purpurogenum
ee. Conidia smooth . . . . . P. rubrum
dd. Colonies without deep red pigment; reverse, dark yellow-green to brown or almost black . . . . P. herquei

Penicillium frequentans Westling (Pl. VIII, d)

Colonies on Czapek's solution agar spreading rapidly, attaining a diameter of 5 to 6 cm in 12 to 14 days at room temperature, broadly zonate, radiately wrinkled in most strains with central area commonly sulcate, thinning at the margin, consisting of a closely woven felt of coarse hyphae either above or below the surface from which abundant crowded conidiophores arise, velvety, but commonly showing limited trailing hyphae, heavily sporing, usually celandine to artemisia green, becoming storm gray in age, in some strains darker, exudate limited, clear to light amber; odor faint, moldy; reverse mostly in shades of yellow-orange near russet to mars brown, but occasional strains appearing light purplish brown; conidiophores short, up to 100-200 x 3-3.5 microns, with walls smooth or finely roughened, and apices enlarged up to 5 microns or more in width; conidial structures forming a crowded, fairly continuous layer over the whole culture with penicilli almost entirely monoverticillate but showing an occasional branch; phialides in crowded clusters numbering

10-12 or more in the verticil, mostly 8-12 x 3-3.5 microns, commonly producing chains of conidia in fairly well-defined columns up to 150 microns or more in length, sometimes splitting in age, in other strains showing conidial columns only loosely parallel; conidia globose to subglobose, comparatively thin-walled, smooth or finely roughened, mostly 3-3.5 microns in diameter.

Penicillium decumbens Thom

Colonies on Czapek's agar slowly spreading, attaining a diameter of 2-3 cm in 12 to 14 days at room temperature, almost velvety in some strains, in others showing a tendency to develop white mycelial overgrowths in central areas, in still others almost floccose and fairly deep up to 1-2 mm but all characterized by loosely interwoven and trailing hyphae bearing short conidiophores, sporulating over the whole colony surface, marginal growth in some strains very thin, largely submerged in zones from 1-3 mm wide, colored in grayish yellow-green shades near gnaphalium to tea green, in older colonies developing surface tufts of sterile secondary mycelium; exudate lacking or inconspicuous; odor distinctive, fragrant, reverse colorless or with a slight greenish cast; conidiophores 50-100 x 2-2.5 microns, with apices slightly enlarged, smooth or finely roughened, borne at successive nodes upon trailing hyphae which in marginal areas of many strains grow stolon-like along the substratum; penicilli almost entirely monoverticillate and only occasionally showing a branch, producing loose columns of conidia up to 100 microns in length; phialides mostly in compact clusters up to 12-15 in number, 7-9 x 2-2.5 microns, sometimes borne at two immediately adjacent levels; conidia elliptical to subglobose, 2-2.5 microns in long axis, occasionally up to 3 microns, smooth, appearing slightly green under the microscope.

Penicillium waksmani Zaleski

Colonies on Czapek's agar growing rather restrictedly, attaining a diameter of 1.5-2 cm in ten days at room temperature, strongly wrinkled and buckled, with central area generally raised 1-2 mm, consisting of a closely-woven basal felt of delicate hyphae, appearing velvety in marginal areas after one week, margin thin and with submerged mycelia usually extending 1 mm or more beyond the aerial growth, medium to light sporulating after one week, in pale blue-green shades near court gray or celadine green, to deep or dark olive gray in older colonies; exudate limited in amount, in small droplets, clear; odor lacking or indefinite; reverse uncolored or in pale peach shades; conidiophores arising from the basal felt as ascending criss-cross rather than erect branches, from very short to 100-200 x 1.5-2 microns, with apices somewhat enlarged up to 2.5-3 microns, smooth-walled or nearly so; penicilli monoverticillate, often appearing singly, sometimes in irregular clusters of 2,3, or more, borne on separate branches and retaining their individual monoverticillate character; phialides in compact of 6-10, mostly 6-8 x 2-2.5 microns, diverging at the tips; conidia globose to subglobose, mostly 2-2.5 microns, occasionally larger with walls delicately roughened, in divergent chains 50-100 microns in length, becoming tangled.

Penicillium charlesii Smith (Pl. VIII, a)

Colonies on Czapek's agar growing rather restrictedly, attaining a diameter of 2 cm in 10 to 12 days, strongly buckled, and wrinkled with central area commonly depressed, consisting of a comparatively thin, close-textured, tough basal felt, appearing velvety or nearly so, medium to light sporing throughout, heavier in submarginal area, in dull green shades near artemisia or lily green, becoming olive gray in age, with growing margin thin, narrow, rarely exceeding 1 mm, white; exudate lacking; odor slight, not distinctive, reverse in dull greenish shades; conidiophores arising from creeping or closely interwoven aerial hyphae, simple or variously branched, occasionally showing a fairly compact group of metula-like branches, each branch bearing a monoverticillate penicillus with conidial chains forming a compact column up to 150 microns or more long, conidiophores variable in length from very short to 100 microns or more by 2-2.5 microns in diameter with terminal area enlarged, occasionally wedge-shaped or more commonly with apices inflated somewhat vesicular about 4-5 microns; walls smooth or nearly so; phialides in compact clusters, roughly parallel, numbering up to 10 or 12 in the verticil, 7.5-9 x 2.2-2.5 microns; conidia ovate to slightly elliptical, roughened, mostly 2.5-3 x 2-2.5 microns, dark green in mass.

Penicillium janthinellum Biourge (Pl. VIII, b)

Colonies on Czapek's agar spreading, attaining a diameter of 5-7 cm in 10 days at room temperature (24°C), forming a tough, closely interwoven felt of fine hyphae, with growing margin broad, with surface growth delicately floccose, unevenly tufted, or in some strains ropy, irregularly wrinkled in central portions and radially furrowed in marginal colony areas, at first white, but in most strains becoming variously colored from the tardy and irregular development of conidial areas, mostly in pale gray to glaucous gray shades and the simultaneous shading of nonfruiting areas to dull buff, orange-red, or in some strains purple-vinaceous shades, azonate or broadly zonate; exudate usually lacking or limited, occasionally abundantly produced, colorless to amber, brownish, or vinaceous; reverse of colonies sometimes colorless, especially in stock cultures after many transfers, but usually in bright shades, in new isolates commonly yellow-green to orange at first, quickly changing to orange-red, maroon or purple-red shades; penicilli typically asymmetric, strongly divaricate with conidial chains divergent or tangled and commonly up to 200 microns in length, abundantly produced in some strains, less abundantly in others, sometimes thinly or evenly distributed over the whole colony but generally more abundant in submarginal areas, borne terminally on ascending conidiophores up to 500 x 3.5 microns, with walls smooth or finely roughened, or on short branches from aerial hyphae commonly 10-50 x 2.5-3 microns, varying in complexity from simple verticils of phialides (appearing monoverticillate) to verticils containing both metulae and phialides, or verticils of unequal length, and occasionally larger structures with metulae and phialides borne upon one or more branches in addition to the main axis; branches variable, ranging from 10-25 x 3-3.3 microns; metulae mostly 10-15 x 2-2.5 microns, but ranging from 7-20 microns in length, with apices more or less vesiculose; phialides diverging, enlarged at the base then tapering abruptly

to fairly long conidium-bearing tips mostly remaining elliptical, but in some strains becoming ovate to subglobose, with ends often apiculate and walls more or less roughened, commonly 3-3.5 microns in long axis.

Penicillium citrinum Thom

Colonies on Czapek's agar growing restrictedly, generally 2 to 2.5 cm in diameter in 10 to 14 days at room temperature typically furrowed in a radial pattern, often conspicuously so, ranging from velvety in most strains, more or less floccose in some, to close-textured and almost leathery in others; conidial production varying from light to abundant in different strains and to some degree depending upon the number of colonies in the culture plate, zonation more or less evident in some strains, not in others; conidial areas in blue-green shades near celandine green at first, becoming artemisia green to lily green at maturity and finally mouse gray to deep olive gray in age, conidium production often occurring late (after 8 to 10 days) and commonly not uniformly throughout the whole colony, generally heaviest in marginal to submarginal areas; abundant exudate in the form of pale yellow to straw colored droplets of varying size usually produced; pronounced mushroom odor in some strains, not marked in others, reverse usually in yellow to orange shades, the agar becoming similarly colored and often assuming a definite pinkish tint; conidiophores arising mostly from substratum, or from aerial hyphae in the deeper colony centers or in floccose strains, mostly 50-200 x 2.2-3 microns, usually unbranched but occasionally bearing one or more branches 25-35 microns in length, smooth-walled throughout; penicilli typically consisting of a terminal group of 3, 4 or occasionally more, somewhat divergent metulae that measure about 12-30 x 2.2-3 microns (apices commonly enlarged to 4-5 microns), each supporting a cluster of 6 to 10 more or less crowded and parallel phialides measuring about 8-11 x 2-2.8 microns, and bearing conidia in parallel chains to produce well defined columns up to 100-150 microns in length; conidia globose to subglobose, mostly 2.5-3 microns but ranging from 2.2-3.2 microns, smooth-walled or nearly so, but often appearing granular when examined in air bubbles.

Penicillium steckii Zaleski (Pl. VIII, e)

Colonies on Czapek's agar growing rather restrictedly, attaining a diameter of about 2 cm in 10 to 12 days at room temperature, consisting of a close-textured, tough basal felt tearing irregularly, with surface velvety or delicately fibrous, plane or irregularly wrinkled or developing shallow radial furrows in marginal areas, often more or less zonate with growing margin 1-2 mm wide, thin, white, shading quickly to dull yellow-greens with the development of conidial structures; colonies medium to heavy sporing throughout, approximately gnaphalium green to pea green, or, in age, approaching storm gray, commonly developing limited, more or less flocculent and often sterile overgrowths in age and showing a marked tendency to develop sectors differing in depth, texture, amount of sporulation; exudate limited to abundant, mostly in small drops, from colorless to very light yellow; odor at first lacking or not pronounced, in age becoming somewhat moldy or sourish; reverse at first colorless or nearly so, often becoming dull yellowish near olive buff within 2 to 3

weeks; conidiophores abundantly produced, arising from the substratum or the basal felt, variable in length but usually comparatively short, rarely exceeding 200 to 250 microns commonly less, by 2.8-3.3 microns, with walls smooth, usually unbranched; penicilli typically biverticillate and consisting of a terminal verticil of 3 to 5 metulae bearing compact up to 150 microns or more in length by 10-20 microns wide, metulae commonly differing in length, mostly 12-15 x 2.8-3 microns but ranging from 10-18 microns long; phialides in crowded compact clusters, parallel, up to 8 or 10 or more in the verticil, mostly 8-10 x 1.8-2.2 microns, uniform in diameter except for short, somewhat narrowed conidium-bearing tips; conidia globose to subglobose, small, about 2-2.5 microns in diameter, with walls smooth or delicately roughened.

Penicillium lanosum Westling (Pl. VIII, h)

Colonies on Czapek's agar attaining a diameter of 2.5-3 cm in 10 to 12 days at room temperature, consisting of a floccose overgrowth arising from a tough mycelial felt, irregularly wrinkled, with central or subcentral colony areas commonly raised, 1-2 mm deep, producing conidial heads most abundantly in marginal zones with central areas white to very light gray, fruiting areas in pale green to glaucous gray shades near dark glaucous gray or court gray; exudate lacking or limited; odor lacking or indefinite; reverse colorless to light yellowish drab shades; conidiophores arising mostly as short branches from aerial hyphae 100-200 microns in length, less commonly from the substratum and ranging from 250-600 x 2.5-3 microns with walls smooth or delicately roughened; penicilli comparatively large, asymmetric, irregularly branched and tending to become divergent, bearing tangled and irregular spore masses up to 50-75 microns in length; branches variable, commonly 10-20 x 2-2.5 microns, occasionally longer, often arising low on the conidiophore and not appearing as an integral part of the terminal penicillus; metulae few in the verticil, about 8-12 x 2-2.5 microns, commonly borne at different levels; phialides borne in clusters of 5 to 10, measuring about 7-8.5 x 2-2.5 microns, definitely constricted at the point of spore origin; conidia globose to subglobose, 2.5-3 microns in diameter with walls finely granular appearing dull gray-green in mass.

Penicillium lanoso-coeruleum Thom (Pl. VIII, g)

Colonies upon Czapek's agar growing fairly rapidly, about 4-5 cm in diameter in 10 to 12 days at room temperature, deeply floccose, up to 5 mm in deepest areas, with surface very uneven, appearing deeply ridged or broadly tufted, azonate or nearly so, with growing margin white, 2 to 3 mm wide, with conidial areas shading from bluish glaucous at the margin to deep bluish gray-green in the denser areas, with blue coloration pronounced; exudate limited, clear; odor not pronounced but moldy; reverse uncolored or in dull yellowish buff shades; conidiophores arising primarily from the aerial felt, mostly 200-600 microns by about 3 microns, sometimes longer, with walls pitted or delicately roughened; penicilli asymmetric, commonly consisting of a terminal verticil of metulae or showing one or more branches with verticils of metulae and phialides and bearing conidial chains in a loosely columnar or tangled mass, sometimes up to 200 microns or more in length, but mostly 10-15

x 2.8-3.3 microns; metulae in verticils of 3 to 5, mostly 10-15 x 2.5-3 microns; phialides few in verticil, about 8-10 x 2-2.5 microns; conidia showing some ellipticity, up to 3.5 or even 4 x 2.5-to 3 microns, less commonly subglobose, smooth-walled.

Penicillium commune Thom

Colonies on Czapek's agar attaining a diameter of 3 to 4 cm in 10 to 12 days at room temperature, floccose, with mass of mycelium about 500 to 700 microns deep, marginal zone white, about 2 mm wide followed by zones of gray-green, court gray, gnaphalium green and pea green becoming olive-gray to mouse gray in age, at times showing few to several shallow radiate furrows, spreading evenly as submerged and aerial hyphae at colony margins and quickly developing into a complex felt of branching hyphae, the resulting felt being rather tough and showing at the margin a trace of fasciculation or ropiness; exudate colorless, limited, and enmeshed in the floccose mycelial mass; odor fairly strong, "moldy", reverse colorless or yellowish, sometimes with traces of rose; conidiophores varying from very short up to 500 x 5 microns in marginal areas of older colonies, with walls finely roughened to coarsely granular in age, bearing penicilli 40-50 microns in length and ranging up to 80 microns; penicilli branched, asymmetrical, characterized by appressed branches and metulae often borne at different levels, conidial chains tending to form compact masses at first but often diverging in age to produce a loose tangled mass, branches variable in dimensions up to 15-20 microns in length; metulae 15-20 x 3-3.5 microns; phialides few in the verticil, usually produced at approximately the same level, measuring 10-12 x 3 microns; conidia elliptical to subglobose, mostly 4-5 microns in long axis, smooth-walled.

Penicillium funiculosum Thom

Colonies on Czapek's agar spreading broadly in most strains, commonly 4.5 to 5.5 cm in 12 to 14 days at room temperature consisting of a fairly tough basal felt, usually with aerial growth loose-textured and varying in depth up to 2 to 3 mm, in most strains showing aerial ropes of hyphae, often large and conspicuous and commonly dominating the colony appearance, in others more or less tufted, especially in central colony areas, and in still others essentially floccose with ropiness evident but strongly reduced; variable in color depending upon the relative amounts of vegetative mycelium and conidial structures and the pigmentation of the underlying agar, in some strains white to pink or flesh shades, in others developing yellow to orange or red colors with some encrustment of aerial hyphae; sporulating irregularly, often heaviest in central and marginal colony areas, conidial areas varying in color, usually in yellow-green shades from pea green through sage green to slate olive or deep grape green to Lincoln Green, but with colors or conidial areas often altered or obscured by pigmented hyphae; exudate lacking or limited in amount, clear or lightly colored; odor lacking or mild, slightly earthy; reverse variable from flesh through pink to deep red, or in some strains orange-brown, usually deeper under areas of heaviest conidial development; conidiophores arising mainly at right angles from funiculose hyphae, often very short, in marginal areas sometimes arising

directly from the substratum, ranging from 100-300 x 2.5-3.3 microns, with walls smooth or nearly so, in some strains lightly colored, mostly simple but occasionally branched in the terminal areas; penicilli of a single terminal verticil of metulae, often of different lengths (the central metula usually longest), not infrequently showing individual metulae rebranched below the level of the phialides, with walls in greenish tints; metulae mostly 5 to 8 in the verticil, about 10-13 x 2.2-2.8 microns, occasionally longer; phialides mostly in verticils of 5 to 7, closely parallel, about 10-12 x 1.8-2.2 microns but in individual strains often longer or shorter; conidia elliptical to subglobose, mostly 2.5-3.5 x 2-2.5 microns, with walls comparatively heavy, smooth or delicately roughened, borne in tangled chains up to 100 microns in length.

Penicillium purpurogenum Stoll (Pl. VIII, i)

Colonies on Czapek's agar growing rather restrictedly, attaining a diameter of 1.5 to 2.5 cm in 12 to 14 days at room temperature, sometimes definitely wrinkled, zonate or azonate, consisting of a yellow to orange-red mycelial felt bearing abundant conidial structures, or of massed conidial heads arising from aerial hyphae or directly from the substratum and superficially appearing velvety, or in some strains tending to become floccose with growing margin white or yellowish from an admixture of encrusted sterile hyphae; usually heavily sporing in central and subcentral areas, in deep yellow-green shades near lily green through deep slate green to dull greenish black; exudate usually limited but in some strains fairly abundant, in orange-red shades; odor indistinct or slightly moldy; reverse in deep red to dark reddish purple shades, often approximating blood red, with surrounding agar similarly colored in somewhat lighter shades; conidiophores arising from the substratum and measuring up to 100-150 x 2.5-3 or 3.5 microns or as branches from aerial hyphae and much shorter, about 40-50 microns, smooth-walled; penicilli typically biverticillate and symmetrical, compact, usually consisting of a single verticil of 5 to 7 or 8 metulae, each terminating in a compact cluster of 4 to 6 parallel phialides bearing short conidial chains; metulae 10-14 x 2.5-3 microns; phialides mostly 10-12 x 2-2.5 microns, lanceolate in form, characteristically tapered; conidia elliptical to subglobose in some strains, sometimes more or less apiculate, mostly 3-3.5 x 2.5-3 microns with walls typically heavy and irregularly roughened, sometimes showing distinct transverse bands, but in some strains almost smooth.

Penicillium rubrum Stoll (Pl. IX, a)

Colonies on Czapek's agar growing restrictedly, attaining a diameter of 1 to 2 cm in 12 to 14 days at room temperature, consisting of a basal felt up to 1 mm deep and conspicuously furrowed in some strains, in others much thinner and almost plane, usually more or less zonate, developing abundant conidial structures throughout the colony or in localized areas, usually heaviest near the colony margin, conidial areas in yellow to gray-green shades near pea green to sage green with nonsporulating or lightly sporulating areas usually showing orange-red coloration from pigmented aerial hyphae; exudate usually limited in amount, in small droplets reddish to bright red in color; odor indistinct; reverse bright

orange-red to cherry-red with surrounding agar colored in lighter tints of the same shades; conidiophores up to 200 microns or more in length by 2.2-3 microns, with walls smooth or occasionally appearing somewhat granular, arising from the substratum or from creeping or aerial hyphae sometimes more or less funiculose; penicilli biverticillate and symmetrical, usually consisting of a terminal verticil of 5 to 10 metulae, measuring about 8-10 or even 12 x 2-2.5 microns; phialides lanceolate with apices tapered in the manner characteristic of the group, in verticils of 5 to 8, mostly 10-12 x 2-2.2 microns, in individual strains longer or shorter; conidia smooth-walled, variable in dimensions, from strongly elliptical 3-3.5 x 2-2.5 microns in some strains, to ovate to subglobose 2.2-2.8 x 2-2.5 microns in others.

Penicillium herquei Bainier and Sartoris (Pl. IX, b)

Colonies on Czapek's agar growing slowly, attaining a diameter of 2 to 3 cm in 2 weeks at room temperature, often somewhat radially wrinkled, azonate or slightly zonate, deeply velvety to almost lanose, consisting of a thin basal felt with conidiophores arising from the felt or directly from the substratum, medium to heavy sporing, in yellow-green shades near tea green through vetiver to Andover green; exudate lacking or limited; odor sometimes lacking or indefinite but usually strong, variable, often suggesting black walnuts, occasionally apples, or in some strains spicy; reverse in dark yellow-green (sometimes almost fluorescent) shades, becoming brown at margins, or occasionally under the entire colony, surrounding agar highly colored in lighter tints of the same shades; conidiophores 200-300 x 3.5-4.5 microns, appearing extremely rough and heavily encrusted when viewed dry under the low powers of a compound microscope, but in liquid mounts often appearing smooth, or again showing a coarse roughening immediately below the penicillus; penicilli comparatively short, regularly consisting of a terminal verticil of metulae bearing clusters of phialides; metulae usually in verticils of 4 to 6 but sometimes up to 8 or 10 commonly measuring 10-15 x 4-4.5 microns somewhat enlarged at the tips; phialides 9-12 x 3-4 microns in clusters of 8 to 12, tapered abruptly to narrow beak-like conidial tubes; conidia elliptical, typically smooth or nearly so, 3.5-4 x 2.2-3 microns, borne in tangled or loosely parallel chains up to 100 microns or more in length.

Penicillum palitans Westling

Colonies on Czapek's agar attaining a diameter of 3.5 to 4 cm in 12 to 14 days at room temperature, ranging from about 300 to 500 microns deep in marginal to submarginal areas up to 1 to 2 cm in colony center, surface appearing granular or mealy but generally not conspicuously fasciculate, often narrowly zonate with shallow to prominent radial furrows, growing margin white, about 1 mm wide, heavily sporing throughout in most strains, at first in greenish glaucous shades through pistachio green to shades of American green or Russian green at maturity, becoming grayish olive in age, often tending to develop limited areas of white sterile overgrowth; exudate clear or light amber abundantly produced in some strains not in others; odor pronounced, moldy; reverse colorless at first, through dull yellow shades to pale brown and finally light purp-

lish brown; penicilli asymmetric, comparatively large, mostly 40-50 microns in length but ranging from 30-70 microns and bearing tangled, divergent, or loosely parallel conidial chains up to 100 microns or more in length; conidiophores mostly 100-200 x 3.5-4 microns in some strains, definitely longer in others up to 300 to 400 microns with walls roughened; penicilli generally showing 1 or 2 branches in addition to the main axis, branches typically appressed and often unequal in the same penicillus, commonly ranging from 15-25 x 3-3.5 microns; bearing metulae in groups of 3-5 mostly 10-15 x 2.8-3.3 microns; phialides usually borne in compact verticils of 5 to 7 measuring 8-10 x 2.2-3 microns; conidia elliptical when first formed, then globose to subglobose, mostly 3.5-4 microns in diameter but ranging up to 4.5 or even 5 microns, with walls comparatively heavy, smooth or nearly so.

Penicillium cyclopium Westling (Pl. IX, c)

Colonies upon Czapek's agar usually growing rapidly, attaining a diameter of 4.5 to 5 cm in 12 to 14 days at room temperature, usually more or less radially furrowed, from 500 to 1000 microns deep, azonate or broadly zonate in age, in some cultures tending to develop limited sterile overgrowths, with margin compact, white, 1 to 2 mm wide during the growing period, often thinning in age, heavily sporing throughout and shading quickly through bluish or green shades in young conidial areas to deeper shades near bluish gray-green, artemisia green or lily green at maturity, with surface typically appearing granular or "mealy", conidiophores arising from the substratum, often crowded into fascicles or tufts, but usually borne more or less separately, exudate lacking in some strains, abundantly produced in others, clear or very faintly colored in pink or orange shades; odor pronounced, "moldy" but difficult to characterize; reverse uncolored or yellowish at first, becoming orange-brown or even purplish in two weeks in most strains, remaining essentially colorless in others; penicilli large, about 50 to 60 microns in length, asymmetrically branched, bearing tangled chains of conidia in irregular masses up to 150 microns long; conidiophores arising from the substratum, mostly 200-400 x 3-3.5 microns, sometimes coarser, with walls typically roughened but in some strains appearing smooth or nearly so; penicillus usually showing one or occasionally more branches, usually 15-30 x 2.5-3.5 microns, often appressed and like the main axis usually bearing 3 to 4 metulae 10-15 x 2.5-3.3 microns, each supporting a verticil of 4 to 8 phialides measuring 7-10 x 2.2-2.8 microns, with apices ending abruptly in conidial chains; conidia mostly subglobose 3.5-5 microns in diameter but with both globose and elliptical conidia observed, the latter commonly ranging from 3.3-4 x 2.5-3 microns, with walls smooth or delicately roughened.

Penicillium puberulum Bainier (Pl. IX, d)

Colonies on Czapek's agar growing more or less restrictedly, attaining a diameter of 3-3.5 cm in 2 weeks at room temperature, with surface velvety to somewhat granular, raised and occasionally almost umbonate in central areas, with submarginal areas radiately wrinkled, azonate during the rapidly growing period but becoming more or less zonate in age and often showing a thin, spreading marginal area up to 1 cm in

width, closely but delicately zonate; growing margin white to light gray-green, 1 mm wide, quickly becoming darker, fruiting areas at first bluish-green near gnaphalium green but shading quickly to slate gray and finally to dark olive-gray, at 2 to 3 weeks commonly showing an area of submerged growth up to 2 mm wide surrounding the colony and bearing scattered penicilli; reverse yellowish to tan to almost brownish black in colony center, with surrounding agar uncolored; exudate lacking or limited in amount, colorless; odor moldy to sourish, strong; conidiophores arising primarily from a tough basal mycelial felt, generally less than 200 microns in length by 3.5-4 microns wide, slightly sinuous with walls more or less roughened; penicilli asymmetric, consisting of a terminal verticil of metulae or of such a verticil with branches and metulae arising from a lower node, often irregularly branched; branches usually 10-20 x 2.8-3.5 microns; metulae usually in groups of 2 to 4, ranging in dimensions from 9-15 x 2.5-3.3 microns; phialides usually in groups of 3 to 5 and measuring 7-9 x 2.5-3.5 microns, with form not distinctive; conidia globose to subglobose, with walls smooth or delicately roughened, mostly 3-3.5 microns in diameter but variable in size up to 5-5.5 microns.

Penicillium martensii Biourge (Pl. IX, e)

Colonies on Czapek's agar growing fairly rapidly, attaining a diameter of 4-4.5 cm in 12 to 14 days at room temperature with central areas commonly raised, and usually marked by radial furrows, but in some strains remaining essentially plane, with growing margins 1 to 2 mm wide, white, with marginal areas narrowly but definitely zonate in most strains, and with surface appearing granular to definitely tufted, heavily sporing throughout, shading from glaucous blue shades in young conidial areas through bluish gray-green shades to dark bluish gray-green, lily green or grayish blue-green at maturity and usually becoming dull gray in age; exudate limited or abundantly produced, clear; odor often pronounced, moldy; colonies in reverse, at first, lightly colored, becoming orange-brown to light maroon, and sometimes showing a purplish shade in age, with the surrounding agar becoming similarly colored in most strains; penicilli comparatively large, mostly 40 to 50 microns in length bearing conidia in tangled chains, forming rather irregular masses up to 150 to 200 microns in length; conidiophores arising directly from the substratum, from 200-400 x 3-3.5 microns, with walls generally smooth or in some strains slightly roughened; penicilli usually showing one or two branches, mostly 15-20 x 3-3.5 microns, in addition to the main axis, often but not consistently appressed; metulae commonly 3 to 5 in the verticil, about 10-22 x 2.4-3 microns; phialides usually in clusters of 4 to 8, measuring 7-9 x 2-2.5 microns with apices somewhat narrowed; conidia smooth-walled, elliptical to subglobose, mostly 3.3-4 x 3-3.3 microns but occasionally up to 5-5.5 x 3-4 microns.

Penicillium granulatum Bainier (Pl. IX, f, g)

Colonies on Czapek's agar growing rather restrictedly, approximately 2 to 3 cm in 12 to 14 days at room temperature, comparatively deep, up to 2 to 3 mm, more or less flocculent with abundant sterile hyphae, white to dull yellow in color, surface irregularly tufted and wrinkled, sporulating lightly and irregularly with conidial areas limited, in pale blue-green

to glaucous green shades, conidial structures sometimes arising individually from the substratum or from the loose aerial growth but more commonly aggregated to form bundles or conspicuous coremiform fascicles from which individual conidial structures tend to diverge in the terminal portions; exudate limited to abundant, clear to pale yellow; odor pronounced in most strains, fragrant, aromatic; reverse usually in dull yellow to orange-brown shades, occasionally appearing greenish in marginal areas; penicilli asymmetric, comparatively large, bearing tangled and divergent chains of conidia up to 50 to 75 microns, occasionally consisting of a terminal verticil of metulae, more commonly showing one or more branches somewhat appressed, with branches and the main axis bearing verticils of metulae and phialides; conidiophores variable in length, from 100 to 200 microns up to very long in coremiform masses, approximately 3.5 to 4 microns in diameter, with walls of conidiophores, branches, and metulae roughened, echinulate; branches variable, commonly 12-30 x 3-3.5 microns; metulae few in the verticil, about 8-12 x 3-3.5 microns; phialides in small crowded clusters, usually about 6-9 x 2-2.5 microns, occasionally much longer; conidia strongly elliptical when young, in age elliptical to subglobose, mostly 3-3.5 microns in long axis but with individual cells frequently larger, smooth-walled.

Penicillium aurantio-virens Biourge

Colonies on Czapek's solution agar attaining a diameter of 2.5-3 cm in 10 days at room temperature, in some strains growing irregularly with some areas or sectors restricted and strongly tufted and others spreading and tending toward lanose or velvety, ranging from 500 microns to 1 mm or more deep, in other strains thinner and usually showing numerous radial lines which first appear as furrows but subsequently develop at ridges due to secondary growth, zonate with margins white, shading from celandine green to lily green or deep bluish gray-green in areas of mature penicilli; odor strong, "moldy"; exudate limited, colorless to very pale yellow, reverse in orange-red shades with surrounding agar becoming similarly colored, sometimes as a conspicuous halo, 1 to 2 cm beyond the colony margin; sporulating abundantly with conidiophores arising from the substratum or as branches from aerial hyphae, commonly 200-300 x 3-3.5 microns, occasionally longer with walls finely roughened, bearing conidial structures of intermediate size characterized by tangled conidial chains; penicilli biverticillate and asymmetric, irregularly branched with metulae and phialides commonly borne at different levels; branches variable in size, commonly 10-15 x 2.5-3 microns, occasionally longer; metulae usually in clusters of 2-4, commonly 10-12 x 2.5-3 microns; phialides usually 6.5-8 x 2-2.5 microns, sometimes longer with abnormal cells occasionally observed, tending to become detached in mounts of older structures; conidia elliptical to subglobose, commonly 3-3.5 or even 4 microns long, smooth-walled.

Penicillium urticae Bainier

Colonies on Czapek's solution agar growing restrictedly, attaining a diameter of 2-2.5 cm in 12-14 days at room temperature, radiately furrowed in most strains, with margins abrupt and with central area often somewhat raised, ranging from 0.5-1 mm deep in marginal areas to 2-

3 mm deep in colony centers; surface distinctly granular in most strains, with prominent fascicles usually produced at least in marginal areas, heavily sporing throughout, approximately gnaphalium green, in some strains less heavily sporing and somewhat floccose, approximately court gray, and in occasional heavily sporing strains appearing darker green near pea green or artemisia green; exudate not produced in some strains, abundantly in others with droplets typically large, clear or nearly so, and often largely embedded in the colony mass; odor distinctive and fragrant in some strains, not pronounced in others; reverse at first dull yellow becoming orange cinnamon to reddish brown shades with agar slightly colored beyond the colony margin; penicilli loosely divergent, comparatively large but extremely variable in pattern and dimensions, commonly 40-50 microns in length but ranging from 20-90 microns, bearing more or less divergent conidial chains up to 50-100 microns in length; conidiophores partly in fascicles, partly simple, undulate or sinuate, with walls smooth commonly 400-500 x 3-4 microns; penicilli variously branched with conidium bearing elements commonly arising at different levels, branches divergent but mostly 15-20 x 3-3.5 microns ranging from 12-30 x 2.8-4 microns; secondary branches when present, mostly 12-15 x 3-3.5 microns, but ranging from 10-30 x 2.5-3.5 microns; metulae comparatively short, mostly 7-9 x 3-3.5 microns, commonly in groups of 2-4; phialides short, 4.5-6.5 x 2.2-2.5 microns, crowded in the verticil, commonly in clusters of 8-10; conidia elliptical or tardily subglobose, mostly 2.5-3 microns in long axis with walls thin, smooth.

Penicillium corymbiferum Westling

Colonies on Czapek's solution agar growing rapidly, attaining a diameter of 4.5-5 cm in 8-10 days, up to 1-2 mm deep in central to subcentral areas, azonate or indistinctly zonate near colony margins, strongly fasciculate, with surface appearing coarsely granular or ridged and with the majority of conidiophores aggregated into clearly defined bundles easily viewed at the colony margin or in radial colony sections, heavily sporing throughout, with growing margin and secondary tufts of vegetative hyphae and developing conidiophores white to pale yellow, passing through yellow-green shades near pea-green to sage-green with the development of mature conidia, and becoming slate-olive in age; exudate generally not pronounced; reverse and agar becoming quickly colored in rich yellow-brown shades, less commonly pinkish or reddish-brown; penicilli comparatively large, mostly 40-45 microns in length bearing conidia in tangled chains up to 100 microns or more in length, asymmetric, comparatively regular in form, consisting of one or two appressed branches in addition to the main axis; conidiophores variable in length from 100-200 microns or more at the margin up to 500 to 1000 microns in the strongly fasciculate central areas, 3.5-4.5 microns in diameter with walls closely and conspicuously roughened; branches commonly 15-25 x 3-4.4 microns, occasionally rebranched; metulae in groups of 4 to 6, measuring 12-16 x 3-4 microns with walls of metulae and branches usually roughened like the conidiophores; phialides numerous, in crowded clusters 6 to 10, mostly 9-12 x 2-2.5 microns with walls apparently smooth; conidia globose to subglobose, mostly 3-3.3 microns in diameter but ranging from 2.5-4 microns with walls smooth.

27. Paecilomyces Bainier

Genus related to Penicillium and Aspergillus, distinguished by phialides short tubular or more or less enlarged, tapering into long conidium-bearing tubes mostly curved or bent slightly away from the axis of the main phialidic cells; phialides variously arranged, partly in verticils and branching systems suggesting Penicillium, partly irregularly arranged on short branchlets, partly arising singly along fertile hyphae; conidia in chains, elliptical, never green.

Paecilomyces varioti Bainier (Pl. X, a)

Colonies on Czapek's agar broadly spreading, low growing, felty, with scattered, floccose aerial mycelium; surface olive, olive-buff or brownish-olive, never true green; reverse colorless; conidiophores arise from aerial or submerged mycelium, freely and irregularly branched, up to 325 microns long; conidial fructification typically in two stages, the branches of the conidiophore bearing a terminal verticil of divergent metulae, with divergent phialides; metulae extremely variable in length; phialides 10-25 x 2.5-4 microns; conidial chains very long divergent, seldom more than five or six in a head; conidia elliptical, smooth, 4.5-6 x 2.5-4 microns.

28. Scopulariopsis (Bainier) Thom

Colonies never green, with aerial hyphae, partly at least, in trailing and anastomosing ropes or fascicles; conidiophores very short or lacking, commonly borne along the fasciculate hyphae; conidial apparatus Penicillium-like or consisting of varying aggregations of branches and phialides scattered along aerial hyphae; at times reduced to single phialides scattered along aerial hyphae; phialides more or less specialized, tapering gradually from a basal tubular section or even the base itself a conidium-bearing apex, or narrowly tubular without tapering, cutting off conidia from the apex by crosswalls; conidia more or less pointed at the apex and truncate at the base with a more or less thickened basal ring surrounding a basal germinal pore, with walls usually thickened and often variously marked or roughened.

Scopulariopsis brevicaulis Bainier

Colonies on sugar gelatin, white at first, then yellowish-brown or chocolate consisting of short closely crowded conidiophores making powdery areas overgrown by loose trailing floccose hyphae and ropes of hyphae, with broadly spreading, indeterminate margin; conidiophores short, 10-30 microns long, arising directly from the submerged hyphae, or numerously and irregularly borne as lateral perpendicular branches of trailing hyphae and ropes of hyphae; conidial fructifications either simple chains, terminating unbranched or in sparingly branched conidiophores in young colonies, or verticillately and irregularly twice verticillately branching systems bearing numerous divergent chains, often 150 microns in length in old colonies; phialides continuous with conidiophores, 12-15 x 4 microns, tapering at the apex; conidia somewhat pear-shaped, slightly tuberculate at the apex, with broad base 6.5-7.5 x 7.5-9 microns,

with basal ring and basal pore, in mass light brown to chocolate, smooth at first, then with thick tuberculate walls.

29. Sporotrichum Link

Hyphae creeping, irregularly branched, but never in whorls, branches repeatedly branched; conidiophores not formed or only as projections from side branchlets; conidia borne laterally and terminally on the hyphae or the branches, usually very numerous, sessile or on spicules, oval or globose, hyaline or brightly colored, usually small.

Sporotrichum epigaeum Brunard var. terrestre Dasz.

Mycelium white forming a velvety turf, short, becoming gray with conidial formation; conidiophores septate, little branched, hyaline, slender, 2.5 microns in diameter; conidia on very slender branchlets, which are forked or trifurcate, each branch budding from its tip a gray globose conidium, 2-3 microns, rarely forming short chains.

30. Sporotrichella Karst.

Hyphae spreading, branching, septate, somewhat erect, bright colored; conidia usually single, long spindle-shaped, hyaline, one-celled, Like Sporotrichum but with spindle-shaped conidia.

Sporotrichella rosea Karst.

Turf spreading, often confluent, pink, fading in age; conidiophores as lateral short branches, simple or twice or thrice divided at the tip; conidia terminal fusoid-elongate, straight, hyaline, eguttulate, 10-30 x 3-4 microns.

31. Pachybasium Sacc.

Hyphae forming a turf, creeping, septate, branched; conidiophores erect, branched; primary branches sterile, ending in long, curved thin hyphal tips; secondary branchlets alternating or standing in almost opposite whorls on the ends of which occur many short flask-shaped terminal branchlets bearing the conidia, globose or elongate, hyaline or bright-colored.

32. Verticillium Nees

Sterile hyphae creeping, septate, branched, hyaline or lightly colored; conidiophores erect, septate, branched; branches of the first order whorled, opposite or alternate; branches of the second order whorled, dichotomous or trichotomous on the branches of the first order; further branching similar; terminal branchlets usually flask-shaped and distinctly pointed at the apex; conidia borne singly on the branchlets, soon falling away, globose, elliptic, ovate, obovate, or short spindle-shaped, hyaline or slightly colored, one-celled.

Verticillium terrestre (Link) Lindau (Pl. X, c)

Colonies pure white, spreading, floccose, consisting of dense, cobwebby, branched hyphae; conidiophores erect; septate, usually with four whorls of branchlets rarely again verticillately branched; conidia formed singly at the tips of the branchlets, globose to elliptical, hyaline, 4.4-5 x 3.5-4.5 microns.

DEMATIACEAE

33. Pullularia Berkhout

Mycelium prostrate, branched, divided into numerous isodiametric cells with rounded side (dematioid); conidia hyaline, oval to elongate, budding from the mycelial cells both laterally and terminally.

Pullularia pullulans (de Bary) Berkhout (Pl. X, e)

Conidia as oval to elongate hyaline cells, budding from brown, branching septate mycelial threads, both terminally and laterally; after abstriction the conidia may continue to multiply by budding and abstriction; mycelial cells later divide into a number of isodiametric cells with rounded sides and thick double wall, filled with oil drops.

34. Papularia Fries

Mycelium at first plentiful and white, later becoming gray and then black; submerged hyphae branched, septate, at first hyaline, later yellow brown; conidiophores hyaline, short, collapsing; conidia lenticular, black by reflected light, yellow-brown by transmitted light with a hyaline rim around the periphery.

Papularia sphaerosperma (Persoon) von Hohn. (Pl. X, b)

Colonies spreading, floccose woolly white on surface, reverse at first white, later yellow to brown, spotted by occurrence of dark brown to black sclerotia; conidiophores prostrate or ascending, very variable in length up to 100 microns, hyaline; conidia borne at the apex in an irregular head on short phialides with inflated bases, lenticular 3 x 9 microns usually 5-7 microns in diameter, smooth, black by reflected light, yellow-brown by transmitted light with a hyaline rim around the periphery.

35. Nigrospora Zimmerman

Hyphae prostrate, at first hyaline, later dark, ultimate branchlets bearing jar-shaped conidiophores either laterally or terminally; conidia solitary, subglobose, smooth, black.

Nigrospora sphaerica (Sacc.) Mason (Pl. X, f)

Both sterile and fertile hyphae creeping, at first hyaline, then dark, sterile hyphae septate, 1.9 microns in diameter, fertile hyphae septate, 4 microns in diameter, much branched, bearing swollen jar-like cells terminally and laterally on which are borne single subspherical, smooth black conidia; conidia one-celled, 11-14 microns in diameter.

36. Cladosporium Link

Hyphae prostrate, septate, on the surface or submerged, conidiophores almost erect, branched and floccose, often forming a turf, olive-colored conidia globose and ovate, at first one-celled then becoming septate by a crosswall, usually greenish, terminal and then pressed aside.

Cladosporium herbarum (Persoon) Link (Pl. X, d)

Turf matted, yellow-green, later black-green; conidiophores erect, little branched, septate, brown or olive-green, 5-10 microns in diameter or various heights up to 1/3 mm; conidia terminal, by the extension of the tip falsely lateral, on short knee-like swellings, single or at times in chains, of various shapes, elongate, oval, and then usually one-celled, or cylindric ellipsoid and then with one to four septa, smoky brown or olive green, slightly constricted at the septa, with finely granulate or spiny wall, of very different diameter and length.

37. Helminthosporium Link

Colonies consist of loose or dense hyphae, regularly or irregularly floccose, brown to black with strict or spreading margin; conidiophores usually in groups, erect and straight, sometimes reclining, usually unbranched, only seldom with short side branches, septate, geniculate at points below the conidia, brown, green-brown to black, transparent or opaque; conidia terminal or lateral on the geniculations, elongate, cylindrical, clavate or obclavate, smooth, mostly rounded at both ends, or sometimes pointed at the base or at both ends, straight or curved, with more than four crosswalls, dark brown, green-brown to black, often with the end cells lighter colored.

Helminthosporium sativum Pammel, King and Bakke (Pl. XI, a)

Colonies at first white, becoming brown with spore production; conidiophores fasciculate, 150-180 microns long, 6-10 microns in diameter, dark reddish-brown, conidia straight or curved, tapering toward both ends, ends rounded, olivaceous, 90-130 x 15-20 microns, with seven to fourteen cells.

38. Alternaria Nees

Sterile hyphae prostrate, septate, conidiophores single or in groups, erect, mostly unbranched, short, conidia inverted club-shaped, mostly elongate at the tip, muriform in the lower portion, dark-colored, lighter at the tip, borne in long, usually simple chains.

Alternaria tenuis Nees (Pl. XI, b, c)

Conidiophores short, septate, unbranched or branched, brown-green; conidia inverted club-shaped, in chains, muriform with three to five crosswalls constricted at the outer walls, olive-green or brownish-black, very variable in size and shape, 30-36 x 14-15 microns.

STILBACEAE

39. Graphium Corda
(Pl. XI, d)

Coremia cylindric, clubbed or with a terminal head, dark, rigid; somewhat paler at the tip; conidia oval or elongate, hyaline often enclosed in mucus, one-celled. Coremia formed quite readily in culture particularly around edge of plate and up onto the sides; grayish brown vegetative mycelium was somewhat appressed, often colony reverse was brown also.

TUBERCULARIACEAE

40. Fusarium Link
(Pl. XI, f)

Conidial layer cushion shaped or somewhat extended without a definite limit; conidiophores branched; conidia terminal, simple, spindle or sickle shaped, many-celled with indistinct crosswalls.

Fusarium neoceras Wollenweber and Reinking

Microconidia single or in false heads, not in chains, one-celled, oval spindle-shaped, seldom two-celled, exceptionally three-celled, later scattered as dust in mycelium; macroconidia in sporodochia and pionnotes, brownish white-cream to incarnate, at times becoming flecked with violet or blue tones, and varying in concentric zones of the stroma, and laid on it in rings, straight or slightly curved, tapering at both ends, slightly constricted at tip, with ten-pin to slightly pedicellate base, three, very seldom six to nine septate: 0 septate, 5-8 x 2.75-4.5 microns; 1 septate, 14-34 x 3.24-4.4 microns; 3 septate, 32-59 x 3.5-5 microns; 5 septate, 55-67 x 4.5-5.5 microns; 6-9 septate, 70-120 x 4-5 microns. Chlamydospores and sclerotia lacking.

41. Epicoccum Link

Sporodochia spherical or arched, usually very small, usually with a stromatic underlayer of flat or hemispheric cells; conidiophores rising from this stroma usually very short, dark, abstricted from all sides; conidia single at the tips of the conidiophores, globose or ellipsoid, dark, smooth or spiny or knobby, often reticulate on the surface, one-celled at first, becoming muriformly divided at maturity.

Epicoccum nigrum Link (Pl. XI, e)

Sporodochia scattered, punctate, black on a hemispherical, somewhat depressed, black stroma; conidiophores club-shaped, nonseptate dark, 12-14 x 5-7 microns; conidia globose, scarcely stipitate, dark black-brown, finely warted, reticulately wrinkled, at first one-celled, later muriformly divided, 21-25 microns in diameter. Sporodochia produced rarely in culture except where colony came in contact with a contaminating colony on the plate. Vegetative mycelium gray to white, rather vigorous; colony reverse a typical orange to brown, with knots of brownish-orange hyphae produced on the surface of older colonies.

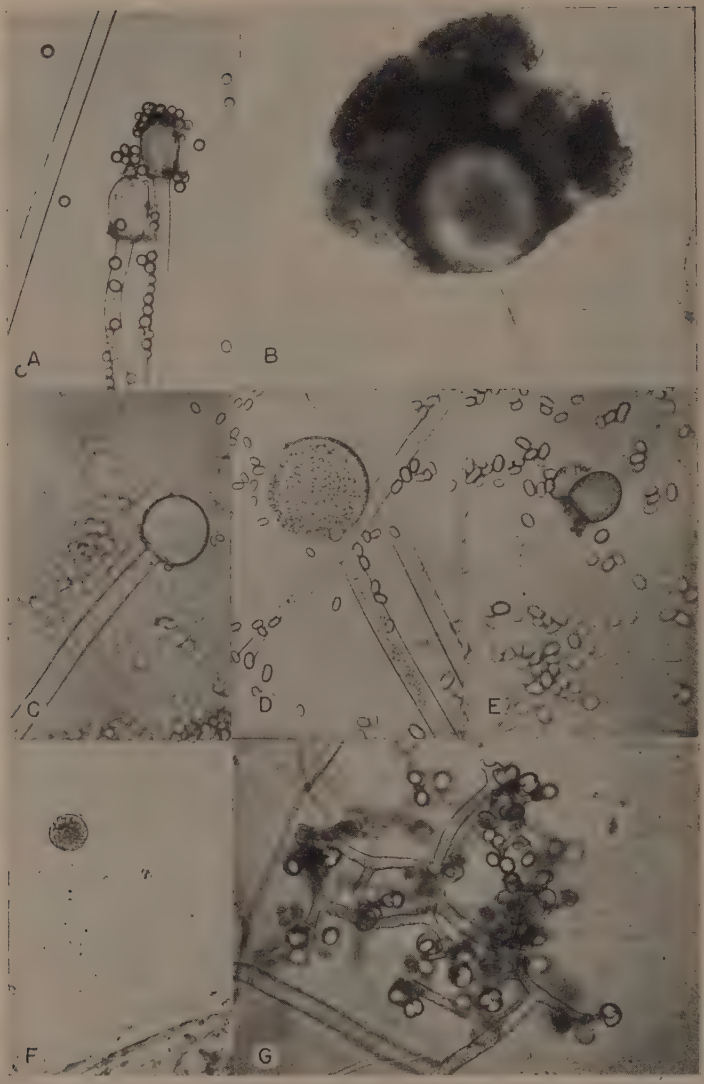

PLATE I. a. Absidia coerulea - columella
b. Rhizopus nigricans - sporangium
c. Mucor varians - columella
d. Mucor hiemalis - immature sporangium
e. Mucor hiemalis - columella
f. Mortierella turficola - sporangium
g. Thamnidium elegans - sporangioles

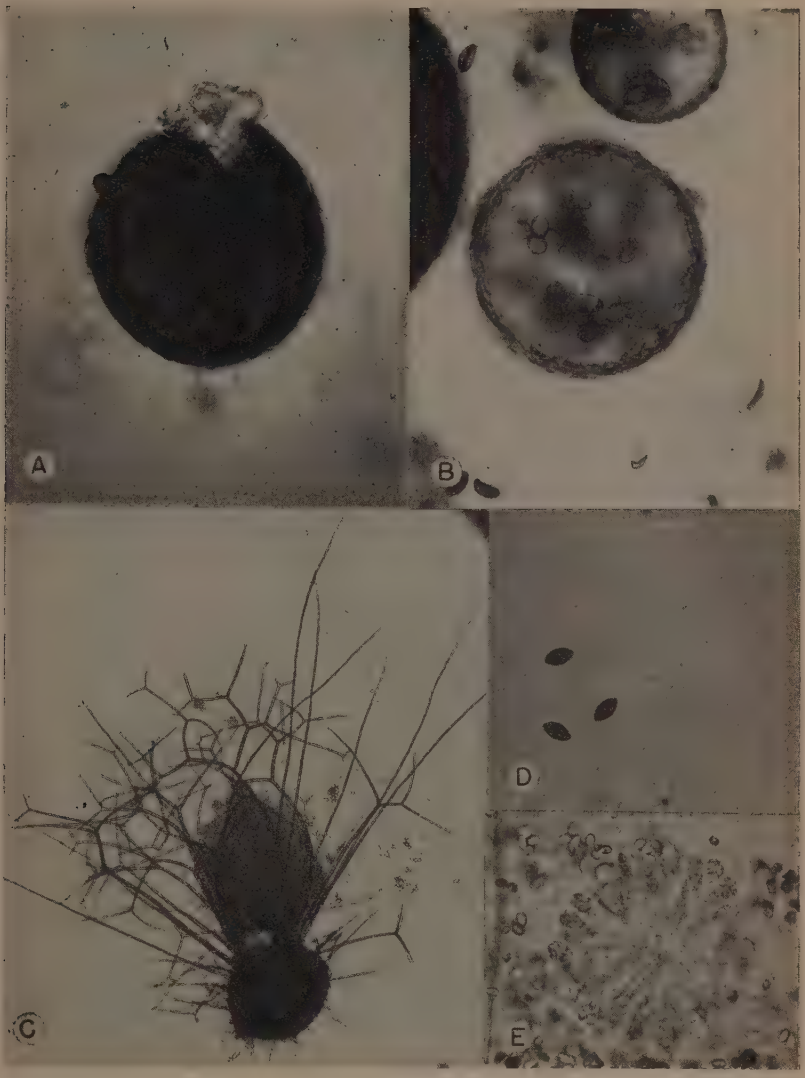

PLATE II a. Thielavia terricola - perithecium
b. Thielavia terricola - sectional view of perithecium
c. Chaetomium dolichotrichum - perithecium
d. Thielavia terricola - ascospores
e. Chaetomium dolichotrichum - asci and ascospores

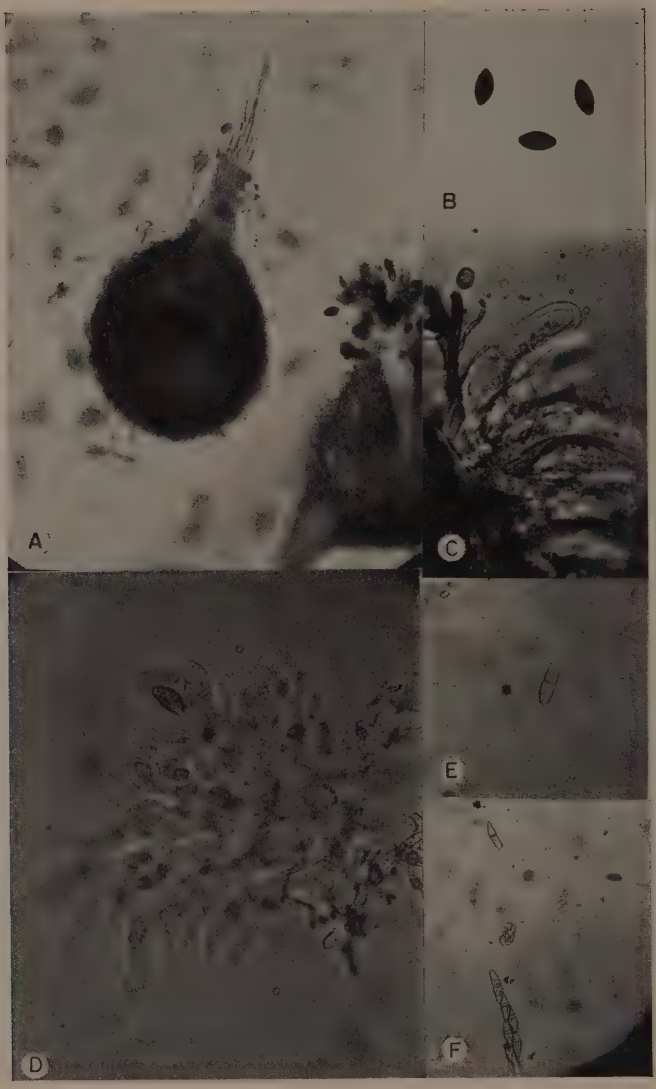

PLATE III a. Melanospora - perithecium
b. Melanospora - ascospores
c. Didymella - asci
d. Melanospora - asci
e. Didymella - ascospores
f. Otthia - ascus

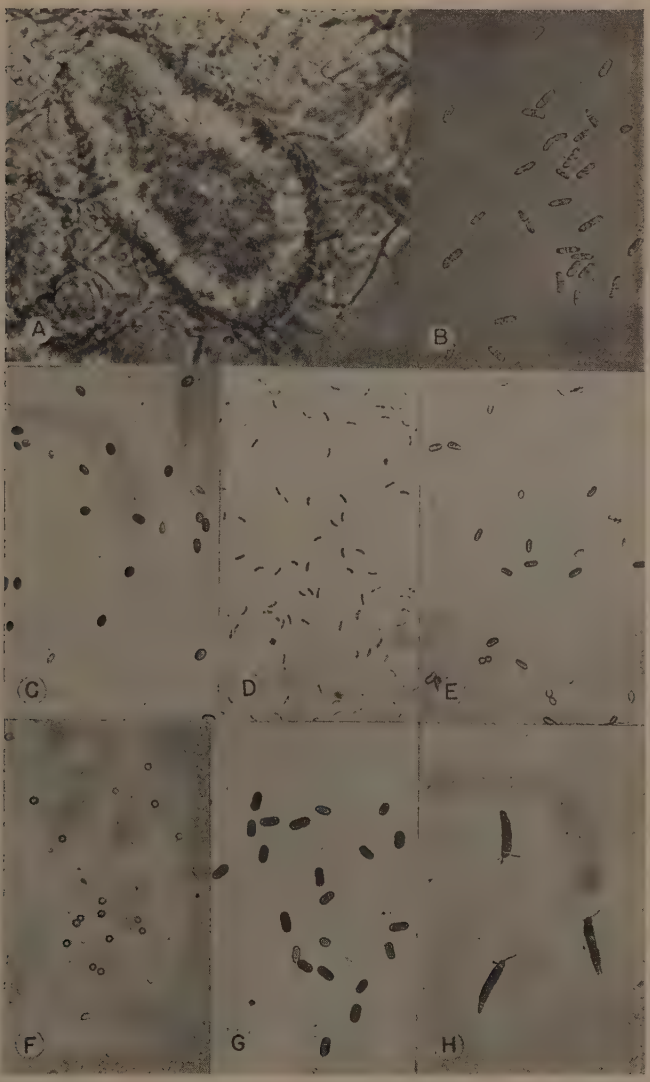

PLATE IV a. Phoma - pycnidium and conidia
b. Ascochyta - conidia
c. Coniothyrium I - conidia
d. Cytospora - conidia
e. Coniothyrium II - conidia
f. Coniothyrium III - conidia
g. Diplodia - conidia
h. Pestalozzia torulosa - conidia

PLATE V a. Geotrichum - conidiophores and conidia
b. Trichoderma lignorum - conidiophore and conidia
c. Hyalopus - conidiophores and conidia
d. Aspergillus versicolor - conidiophores and conidia
e. Aspergillus clavatus - conidiophores and conidia
f. Aspergillus fumigatus - conidiophores and conidia

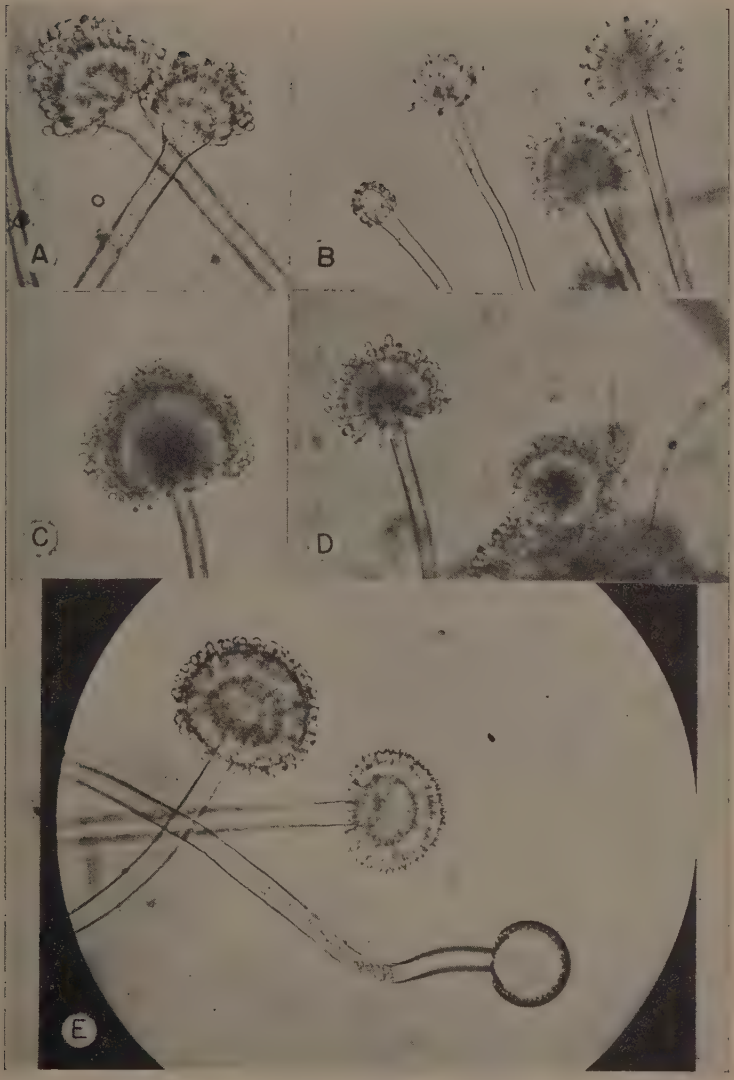

PLATE VI
a. Aspergillus flavus - conidiophores and conidia
b. Aspergillus sydowi - conidiophores and conidia
c. Aspergillus ustus - conidiophores and conidia
d. Aspergillus quercinus - conidiophores and conidia
e. Aspergillus niger - conidiophores and conidia

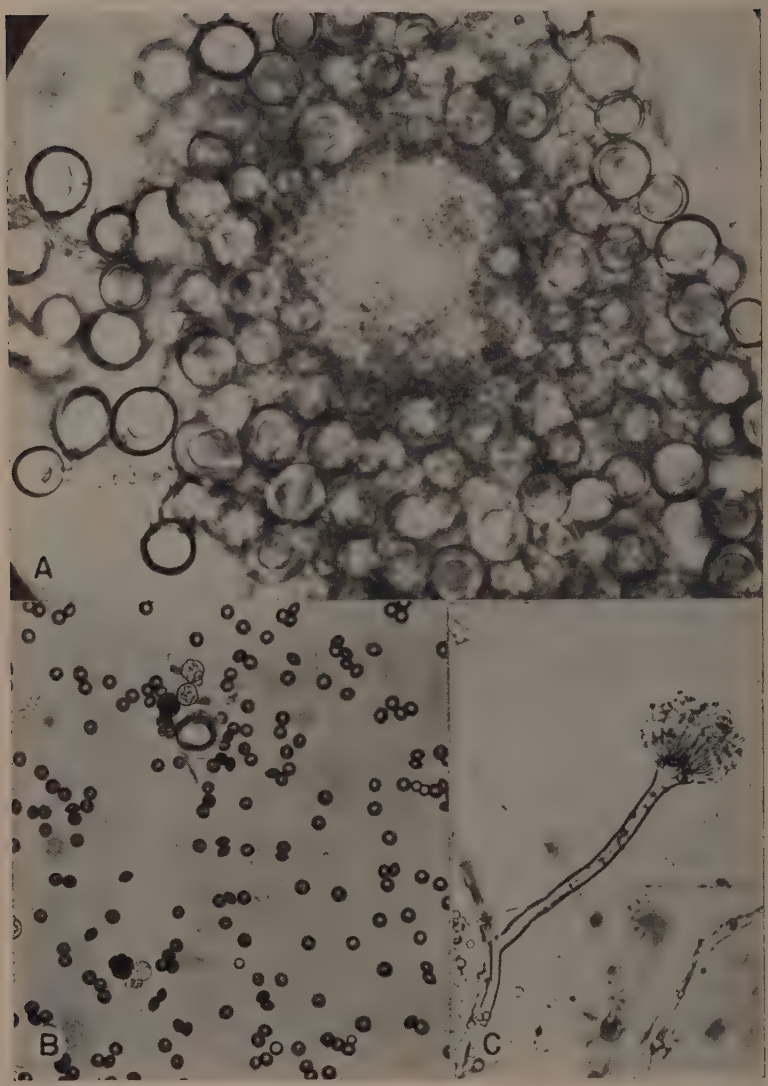

PLATE VII a. Aspergillus nidulans - perithecium
b. Aspergillus nidulans - ascospores
c. Aspergillus nidulans - conidiophore and conidia

PLATE VIII a. Penicillium charlesii - conidiophores and conidia
b. Penicillium janthinellum - conidiophore and conidia
c. Penicillium citrinum - conidiophore and conidia
d. Penicillium frequentans - conidiophores and conidia
e. Penicillium steckii - conidiophores and conidia
f. Penicillium turbatum - conidiophores and conidia
g. Penicillium lanoso-coeruleum - conidiophore and conidia
h. Penicillium lanosum - conidiophore and conidia
i. Penicillium purpurogenum - conidiophores and conidia

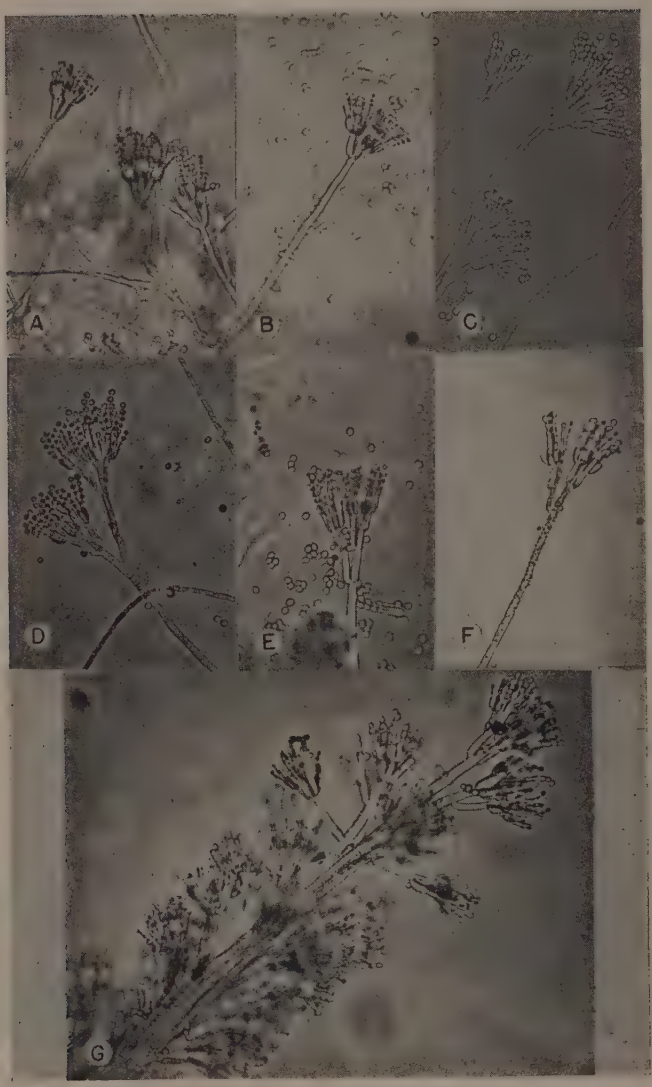

PLATE IX
a. Penicillium rubrum - conidiophores and conidia
b. Penicillium herquei - conidiophore and conidia
c. Penicillium cyclopium - conidiophores and conidia
d. Penicillium puberulum - conidiophore and conidia
e. Penicillium martensii - conidiophore and conidia
f. Penicillium granulatum - conidiophore and conidia
g. Penicillium granulatum - conidiophores and conidia

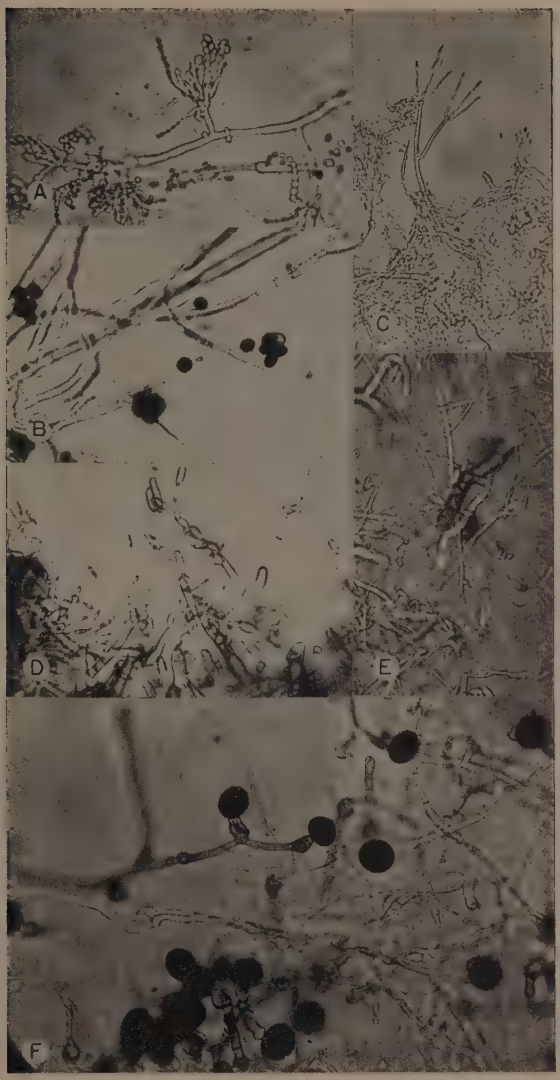

PLATE X
a. Paecilomyces varioti - conidiophores and conidia
b. Papularia sphaerosperma - conidiophores and conidia
c. Verticillium terrestre - conidiophore and conidia
d. Cladosporium herbarum - conidiophores and conidia
e. Pullularia pullulans - conidiophores and conidia
f. Nigrospora sphaerica - conidiophores and conidia

PLATE XI a. Helminthosporium sativum - conidiophore and conidia
b. Alternaria tenuis - conidiophore and conidia
c. Alternaria tenuis - conidia
d. Graphium - portion of head of coremium
e. Epicoccum nigrum - sporodochium and conidia
f. Fusarium - conidia

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THE GROWTH OF GREEN SUNFISH (LEPOMIS CYANELLUS) IN LITTLE WALL LAKE, IOWA[1,2]

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INTRODUCTION

The green sunfish, Lepomis cyanellus Raf., is usually considered a nuisance fish in the Midwest because it rarely reaches catchable size. In the spring of 1948 green sunfish provided some good fishing in Little Wall Lake (R. 24W, T86N, sections 9,10,15, and 16), Hamilton County, Iowa, and many of these fish taken by anglers were seven to nine inches long. The present report is based upon data collected from 467 specimens taken during the period June, 1948 to April, 1950.

Little Wall Lake is a shallow, eutrophic, prairie lake with an area of approximately 230 acres (9). The lake bed is relatively flat, and although some 75 per cent of the lake was 5 feet or more deep in the spring of 1948, the maximum depth recorded was only slightly more than 6 feet. The lake was dry during the period 1936-1941, but recovery was nearly complete when restocking for a largemouth black bass-bluegill management program was begun in 1945. Aquatic vegetation had become so dense by 1948 that fishing was virtually impossible in all but a few limited areas. The fish known to have been in the lake at that time included northern black bullheads, Ameiurus m. melas (Raf.); northern bluegills, Lepomis m. macrochirus Raf.; green sunfish; largemouth black bass, Micropterus salmoides (Lacépède); northern fathead minnows, Pimephales p. promelas Raf.; bluntnose minnows, Pimephales notatus (Raf.); and a few yellow perch, Perca flavescens (Mitchill); black crappies, Pomoxis nigromaculatus (LeSueur); and common white suckers, Catostomus c. commersoni (Lacépède).

Pronounced thermal stratification was not evident in the lake during

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[2]A portion of a dissertation submitted to the Graduate Faculty of the Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy, December, 1950. The author wishes to acknowledge the patient guidance, assistance, and encouragement given by Dr. Kenneth D. Carlander, other members of the Division of Science, Iowa State College, and fellow graduate students during the conduct of this research.

[3]On leave of absence and with the National Science Foundation, Washington, D.C.

the summer. Reduced water level, extensive masses of decaying vegetation, and prolonged periods of snow covering the ice produced critically low oxygen concentrations in Little Wall Lake for approximately a week during mid-February, 1949. Dissolved oxygen concentrations approached zero in the slightly shallower and more heavily vegetated near-shore areas several days before this critical condition was observed in the deeper mid-lake regions. Runoff melt-water from the adjacent watershed had somewhat alleviated the oxygen deficiency at the near shore observation sites by the time depletion conditions were noted at the mid-lake stations. In spite of the short duration of the oxygen depletion period, relatively few individuals from the large populations of bullheads, green sunfish and fathead minnows survived the winterkill conditions. Other fish populations were eliminated completely. Reproduction in the surviving species was good during the following summer. A more severe oxygen deficiency developed in early February, 1950, when dissolved oxygen could not be detected at any of the observation stations for more than four weeks. No green sunfish and only a few black bullheads and fathead minnows are known to have survived the 1950 winterkill.

Several methods (including angling, common sense minnow seine, basket trap, experimental gill net and hoop net) were employed in the collection of specimens. Linear measurements were made to the nearest millimeter and weights were recorded in grams. Scales for the growth study were selected from an area below the lateral line, surrounding and including the point where the posterior tip of the left pectoral fin touched the second and third rows of scales, when the fin was pressed against the body. The largest and best formed scales were mounted dry between microscope slides and were examined at a magnification of 42 diameters on a micro-projector. Scales were read five times, to eliminate inconsistencies. Scale radius was measured between the focus and the anterior margin of the scale.

The methods of collection of fish all seemed to be somewhat selective of different sizes of fish (Table 1). In general, angling, gillnets, and hoopnets seemed selective of the larger fish, except for the July 28 angling sample. Minnow seines and wire basket traps probably selected the smaller fish but the samples taken with these gear are believed to be more representative of the true population.

VALIDITY OF THE SCALE METHOD

Creaser (4), Van Oosten (13), and others have given excellent reviews of the literature concerning the use of the scale method for determining age and growth of fish. Van Oosten (13) and Hile (6) listed criteria for use in testing the validity of annuli as true year marks. Hubbs and Cooper (7) demonstrated the validity of the scale method for age determination in green sunfish. The general validity of the scale method is believed confirmed for green sunfish in Little Wall Lake by the following observations:

(a) Larger green sunfish had more annuli than small individuals. Such a large majority of the fish examined belonged to one year class, however, that length-frequencies gave little clue to actual ages.

TABLE 1

Sizes of green sunfish taken by various gear and at various dates, Little Wall Lake, Iowa.

Date	Method	Standard lengths in millimeters			1946 year class Standard lengths		
		No.	Mean	Range	No.	Mean	Range
June 2-3, 1948	Angling	37	116.8	74-179	33	113.3	74-146
June 16-19, 1948	Angling	3	97.7	79-134	3	97.7	79-134
June 23-30, 1948	Trap	32	72.6	45-128	31	73.0	45-128
June 23-26, 1948	Gillnet	21	92.3	79-138	18	89.1	79-115
June 26, 1948	Seine	26	69.8	55-98	26	69.8	55-98
July 7, 1948	Trap	1	45.0	-	-	-	-
July 7-12, 1948	Gillnet	30	102.6	80-169	26	97.6	80-135
July 17, 1948	Trap	57	64.0	52-90	57	64.0	52-90
July 17, 1948	Gillnet	13	121.4	71-180	11	113.0	71-146
July 28, 1948	Trap	116	71.1	51-143	116	71.1	51-143
July 28, 1948	Angling	26	81.0	65-127	26	81.0	65-127
Aug. 3, 1948	Trap	4	108.5	81-130	4	108.5	81-130
Aug. 3-7, 1948	Gillnet	2	97.5	85-110	2	97.5	85-110
Aug. 7, 1948	Hoopnet	4	111.0	102-123	4	111.0	102-123
Aug. 17, 1948	Hoopnet	7	141.7	115-177	6	123.6	115-164
March 28, 1949	Winterkill	33	117.8	64-200	27	103.0	64-160
July 22, 1949	Seine	36	115.4	104-121	36	115.4	104-121
July 29, 1949	Seine	12	15.3	12-23			
Feb.-April, 1950	Winterkill	5	120.0	43-141	4	139.2	135-141

(b) The dominant group of fish had 2 annuli in 1948 and 3 in July, 1949 indicating that these fish were of the 1946 year class and had added an annulus between the 1948 and the 1949 collections. The winterkill fish had not yet formed a new annulus. The June, 1948 collections indicated that the new annulus had been formed probably in May.

(c) Winterkill survivors collected in late July, 1949, showed a pronounced increase in growth rate over that exhibited by the year-class in previous years. The calculated growth increments for earlier years of life were typical of those shown by certain slow growing individuals of the same (1946) year-class taken in collections made prior to the winterkill. Victims of the 1950 winterkill were also members of the 1946 year-class, and they, in turn, exhibited indications of similar earlier growth increments as well as much increased growth in the calendar year 1949.

FALSE ANNULI

The scales of many of the green sunfish had marks which were less distinct or complete than the typical annuli and were interpreted as being false annuli. These marks did not seem to be consistently attributable to any one factor. More than 50 per cent of the specimens taken from the 1944 and 1945 year-classes possessed one or more false annuli on their scales. Not more than 10.5 per cent of the 1946 year-class individuals taken in pre-winterkill collections had developed false year-marks in

any one year of life. Seventy-five per cent of the winterkill survivors from the same year-class, however, produced a false mark during the period of rapid growth in the summer of 1949.

Some biologists have suggested that false annuli are more common in fast or slow growing populations than in those exhibiting more average growth rates. Certain green sunfish in the present study, particularly those who experienced the excellent growth in the summer of 1949, seemed to conform to this premise. Twenty-five per cent of the specimens in that group, however, did not produce false marks, and in several cases these individuals had grown more than those who did show the marks. In many cases among fish from all year classes the false check appeared to have been formed soon after growth started in the spring, but in other instances the cessation-of-growth mark seemed to have been developed toward the end of the growing season regardless of how great an increment had been produced previously.

Hubbs and Cooper (7) reported the false marks in Michigan green sunfish as being spawning checks. Several Little Wall Lake specimens collected in 1948 were in spent condition when taken and had formed a false annuli previously, so that the time of the two phenomena could have coincided. A few specimens taken earlier in July of the same year had formed current accessory marks but were not yet in spawning condition, the sex organs of one individual being so undeveloped that the investigator was unable to determine the sex. Supernumerary marks were present on the scales of both males and females.

BODY-SCALE RELATIONSHIP

Although for the purposes of calculating growth it is usually assumed that the growth of the scale and the length of a fish are directly proportional, it is often possible to make more accurate computations by determining the mathematical relationship between the body and scale.

When standard length data from the present study were grouped in 5-millimeter intervals and tested for sex-related differences in body-scale ratio, no consistent differences were noted.

The best straight line (Line B of Fig. 1) representing the body length-scale radius data of Table 2 was calculated by the least squares method:

$$L = 4.75 \text{ mm.} + 1.176818\ S$$

where L = standard length (mm.), and
S = anterior length (mm. x 42)

Using the same method, the data were also fitted with a curvilinear regression line (Line A of Fig. 1) represented by the third degree polynomial:

$$L = 10.281 \text{ mm} + 0.776459\ S + 0.00655259\ S^2 + (-0.00002897)\ S^3$$

Since the linear regression line did not appear to fit the data so well by visual inspection as did the curvilinear regression line, the sums of squares of deviations of the data from the lines were calculated to determine mathematically the "goodness of fit". The resultant values were:

Curvilinear regression line, $\Sigma\ (L-Lp)^2 = 16.14$
Linear regression line, $\Sigma\ (L-Lp)^2 = 55.22$

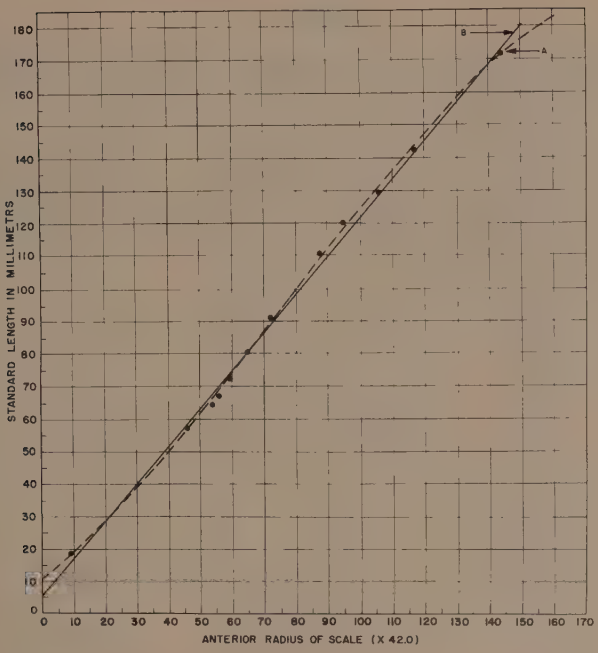

Fig. 1. Body-scale relationship of Little Wall Lake green sunfish. The curvilinear regression line is designated as A, and the linear regression line as B. Dots represent the average body-scale measurements in Table 2.

The pronounced difference between the two values indicates that the curvilinear regression line is the better mathematical fit to the data. Further curvilinear correction to reduce the error remaining after the third degree polynomial calculation is believed unnecessary from a practical viewpoint.

Growth histories were calculated for each specimen using first the linear regression and then the curvilinear regression formula in an effort to determine whether the increase in accuracy obtained by calculation beyond the straight line was of practical importance. When compared, the resulting computed lengths were found to vary by less than 3.5 per cent except for the 1944 year class (Table 3). The simpler straight line relationship was therefore considered most suitable for practical use. The greater differences between the growth values calculated for the 1944 year class are believed due to the lack of sufficient large specimens for the computation of the formulae.

TABLE 2

Average body lengths and scale measurements for size groups used in calculating the body-scale relationship of 459 green sunfish (measurements in millimeters)

Average standard length	Average scale radius (x 42.0)	Average L/Sc ratio	Number of specimens	Computed lengths*	
				Linear	Curvilinear
18.0	8.9	2.022	12	15.2	17.7
57.4	46.4	1.237	40	59.4	57.5
64.5	53.0	1.217	52	67.1	65.5
67.8	55.9	1.213	63	70.5	69.1
72.8	59.1	1.232	60	74.3	73.1
80.2	64.8	1.238	65	81.0	80.2
90.8	71.6	1.268	38	89.0	88.8
110.5	87.6	1.261	45	107.8	109.1
120.2	94.9	1.267	29	116.4	118.2
129.8	105.9	1.226	18	129.4	131.6
142.8	117.3	1.217	19	142.8	144.8
172.0	143.7	1.197	18	173.9	173.3

*Linear $L = 4.75$ mm. $+1.176818\ S$
Curvilinear $L = 10.281$ mm. $+ 0.776459\ S + 0.00655259\ S^2 - 0.00002897\ S^3$

TABLE 3

Difference in standard lengths of green sunfish when calculated on the basis of linear and curvilinear regression formulas (lengths and deviations in millimeters)

Year class	Number of specimens	Average standard length at capture	Lengths determined by curvilinear regression calculations at annulus				Deviation of linear from curvilinear regression values at annulus			
			1	2	3	4	1	2	3	4
1947	1	45.0	33.0	-	-	-	-1.0	-	-	-
1946	430	86.8	26.8	65.2	70.4*	-	-0.4	+1.1	+2.5	-
1945	7	162.0	37.3	93.3	142.0	-	+0.7	-1.9	-3.0	-
1944	7	182.7**	38.1	96.0	138.4	174.7	-2.2	-3.9	-2.3	-0.4

*Based on measurements of 40 specimens.
**Lengths at capture were beyond the reliable portion of the curvilinear regression line.

TABLE 4

Factors for the conversion of standard to total lengths of 426 Little Wall Lake green sunfish.

Standard length in mm.	No. of specimens	Conversion factor
11-20	10	1.259
21-30	2	1.231
-	-	-
41-50	1	1.222
51-60	37	1.242
61-70	111	1.240
71-80	88	1.234
81-90	50	1.250
91-100	16	1.201
101-110	17	1.218
111-120	43	1.222
121-130	21	1.205
131-140	9	1.199
141-150	11	1.198
151-160	4	1.181
161-170	3	1.201
171-180	3	1.194
Non-weighted average	-	1.218

RELATIONSHIP BETWEEN STANDARD AND TOTAL LENGTHS

Conversion factors for standard and total body lengths were calculated for each 10-millimeter size group of the green sunfish taken in the collections (Table 4). Examination of the length conversion factors for males and females failed to reveal consistent sex-related differences. Although considerable irregularity was noted, the data indicated that the caudal fin grew at a relatively slower rate than the body as the individuals increased in length.

GROWTH ANALYSIS

Growth histories calculated on the basis of the linear body-scale relationship formula are presented in Table 5. Increments of growth attained in each year of life by the 1944 and 1945 year-classes were similar. Although the calendar year 1946 appears to have been a good year for reproduction and for survival of the young, the 1946 year-class young-of-the-year grew less than members of other year-classes did in their initial year of life probably because of the greater population and resultant competition. This retarded growth rate was characteristic of the 1946 year-class until the 1949 winterkill occurred and the population density was greatly decreased (Fig. 2).

TABLE 5

Summary of the average calculated standard length and length increments for each year-of-life in Little Wall Lake green sunfish (Number of specimens in parenthesis)

A

Year class	Calculated length (mm.) at end of year-of-life*				
	1	2	3	4	5
1949	43.0 (1)**	-	-	-	-
1947	32.0 (1)	-	-	-	-
1946	26.4 (430)	66.3 (430)	85.0 (67)	139.2 (4)**	-
1945	38.0 (7)	91.4 (7)	139.0 (7)	189.0 (1)	-
1944	37.5 (8)	94.2 (8)	132.6 (8)	172.0 (8)	193.5 (4)
Weighted grand average	29.1 (447)	67.2 (445)	94.3 (82)	163.2 (13)	193.5 (4)
Corresponding total lengths in inches	1.40	3.23	4.53	7.67	9.09

B

Year class	Annual increments in millimeters				
1949	43.0**	-	-	-	-
1947	32.0	-	-	-	-
1946	26.4	39.9	21.2	67.7**	-
1945	38.0	53.4	47.6	29.0	-
1944	37.5	56.7	38.4	39.4	15.8
Weighted average annual increment	29.1	40.4	25.1	47.3	15.8

*Specimens collected prior to annulus formation in 1949 and 1950 were promoted in age on January 1.
**Specimens collected one year after the winterkill of 1949.

Too few sexed specimens were available to adequately determine sex-related growth rate differences in the 1944 and 1945 year-classes. The male fish in the 1946 year-class, however, maintained a slight but consistently greater rate of growth than females in each year of life (Table 6). Hubbs and Cooper (7) reported males exhibiting faster growth in Michigan green sunfish and found the sexual dimorphism in size to increase in the third and fourth summers of life and thereafter.

An interesting difference was noted between the growth history of the 1949 winterkill victims (the March 28, 1949 collection, Table 7) and that of the survivors collected in July, 1949 and April, 1950. All survivors of the 1949 winterkill taken in July of the same year exhibited a previous history of slower growth than that shown by the individuals collected at random from among the recently killed green sunfish at the end of the winterkill on March 28. Those who survived the 1949 winterkill but who died as the result of similar, although more severe, conditions in the late winter of 1950 were specimens who had also achieved slow growth in earlier years but had made an excellent increase in increment in 1949. It would appear from the limited data that the slow growing individuals were possibly able to withstand winterkill conditions better than the more rapidly growing specimens.

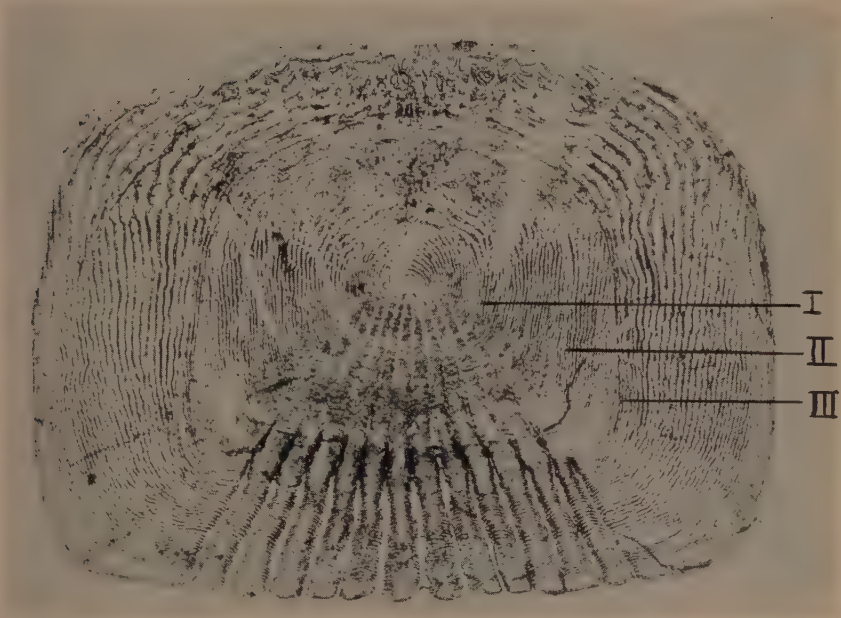

Fig. 2. Photomicrograph of a scale from a 1946 year class. Little Wall Lake green sunfish collected July 22, 1949. Increased current year growth is indicated beyond the third annulus.

The growth of the average green sunfish in Little Wall Lake was rather slow the first couple of years of life and then very rapid in later years, compared to the growth reported for green sunfish from other waters (1, 2, 3, 5, 7, 8, 10, 11, 12).

TABLE 6.

Comparison of calculated standard lengths in millimeters of male and femals green sunfish of the 1946 year class.

	Mean lengths at each annulus (number of specimens in parentheses)		
	1	2	3
Males	26.7 (237)	68.8 (237)	74.9 (25)
Females	25.7 (156)	61.3 (156)	70.0 (11)

TABLE 7

Annual increments of the 1946 year class green sunfish collected before and after the partial winterkill of 1948-49

Collected	No.	Average increment in millimeters in year of life				Growth since last annulus
		1	2	3	4	
June 2-Aug.17, 1948	363	26.6	40.1	-	-	
March 28, 1949	27	28.2	45.5	29.3	-	
July 22, 1949	36	22.9	34.2	15.9	-	42.4
April 1-3, 1950	4	23.2	34.3	14.0	66.7	-

Analysis of variance and tests of significance for differences between March and July, 1949 samples

	No. of fish	Degrees of freedom	Mean length	Sum of squared deviations	Error of mean	t
Lengths at first annulus						
March	27	26	28.222	1310.7		
July	36	35	22.944	285.9		
		61	5.278	1606,6	1.306	4.04
Lengths at second annulus						
March	27	26	45.519	3312.7		
July	36	35	34.139	464.3		
		61	11.380	3777.0	2.003	5.68
Lengths at third annulus						
March	27	26	29.556	7678.7		
July	36	35	15.861	278.3		
		61	13.695	7957.0	2.908	4.71

LENGTH-WEIGHT RELATIONSHIP AND COEFFICIENT OF CONDITIONS

The length-weight relationship of specimens from this study (Table 8, Fig. 3) when computed in the manner reported by Hile (6) was found to be expressed by the equation:

$$\text{Log } W = -4.76800 + 3.16876 \text{ Log } L$$

where W = weight in grams
and L = standard length in millimeters

indicating that weight increases at a rate somewhat in excess of the cube of the length.

Coefficient of condition, K, was determined by the equation:

$$K = \frac{W \times 10^5}{L^3}$$

where W = weight in grams
and L = standard length in millimeters

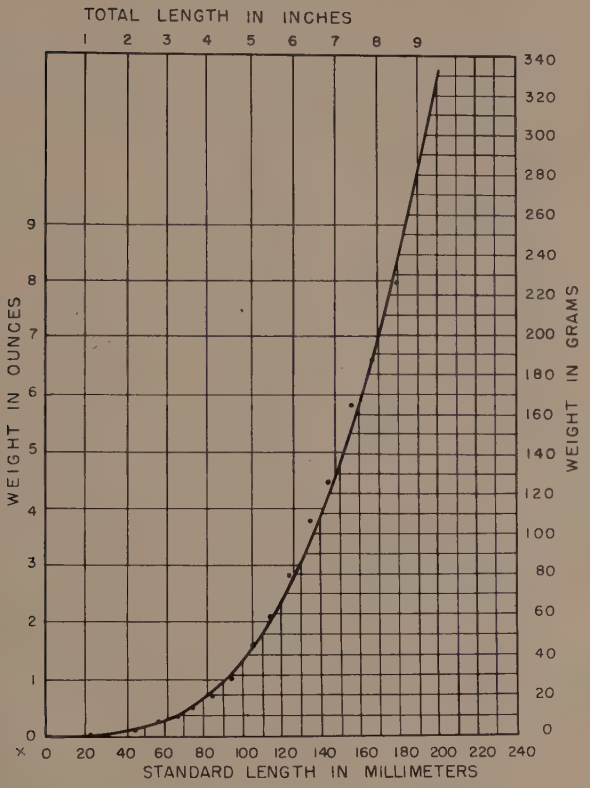

Fig. 3. Length-weight relationship of Little Wall Lake green sunfish. The dots are based on the average standard lengths and actual weights of Table 8.

Since K is a means of expressing the relative plumpness of a fish, a relatively low value of K in comparison with other individuals of the same species represents poorer condition of the specimen. A general trend of increased plumpness in green sunfish with increase in length is suggested by the data (Table 8). No consistent sex-related trends were observed when condition factors of males and females were computed independently.

REPRODUCTION

In 1948 spawning began in early June and continued through mid-August, with ripe and recently spent fish taken throughout the period. The only year-old fish examined, a 45 millimeter male taken July 7 was fully mature with ripe milt. Less than one per cent of the 212 males and only four per cent of the 145 females examined in their third summer were sexually immature and all older green sunfish were mature.

TABLE 8

Length-weight relationships and K factors of 416 green sunfish based on actual lengths and weights, both sexes included. (Lengths in millimeters)

Standard length group	Average standard length	Average weight in grams	Calculated* weight in grams	K	Number of specimens
21-30	22.7	0.37	0.34	3.163	2
41-50	45.0	3.00	2.96	3.291	1
51-60	57.5	6.46	6.43	3.398	37
61-70	66.3	9.65	10.09	3.300	111
71-80	74.6	13.50	14.66	3.254	88
81-90	84.7	19.80	21.92	3.267	50
91-100	95.2	27.60	31.75	3.202	16
101-110	105.9	44.05	44.50	3.696	17
111-120	115.3	58.50	58.27	3.826	43
121-130	125.2	79.36	75.64	4.048	21
131-140	134.7	106.60	95.38	4.363	9
141-150	144.2	126.09	118.30	4.206	11
151-160	156.0	164.50	151.80	4.333	4
161-170	167.0	187.30	188.48	4.021	3
171-180	178.6	226.00	233.20	3.896	3

*Calculated by means of the equation Log W = 4.76800 + 3.16876 Log L.

SUMMARY

A study of the growth characteristics of green sunfish in Little Wall Lake, Iowa, was initiated in 1948 with further collections of specimens being made through early 1950. This shallow prairie lake developed winterkill conditions in the winters of early 1949 and 1950. Observations supporting the validity of the scale method for use in determining age and growth of Little Wall Lake green sunfish are given. The mathematical relationship between standard body length (L) and the anterior radius (S) of the magnified (42X) scale was described better by a curvilinear regression than by a linear regression line, but the increase in accuracy resulting from curvilinear correction of the body-scale relationship was not of sufficient magnitude to warrant use of the curvilinear regression formula in routine practical growth studies of green sunfish. Factors for converting standard to total body lengths are given.

Individuals of the 1946 (dominant) year-class grew at a relatively lower average rate prior to the winterkill than did those fish in other year-classes, but the winterkill survivors grew at an accelerated rate in 1949. There was some suggestion from the data that slower growing green sunfish were possibly better able to withstand limited winterkill conditions than rapidly growing individuals. False annuli were found on the scales of many specimens but their development could not be consistently attributed to any one causative condition.

The mathematical relationship between the standard body length (L) in millimeters and the weight (W) in grams for all data combined was found to be adequately expressed by the equation:

$$\text{Log } W = 4.76800 + 3.16876 \text{ Log } L$$

Coefficients of condition (K) in green sunfish increased irregularly with increase in length.

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USE OF ACTIVATED GLYCEROL DICHLOROHYDRIN FOR ESTIMATING VITAMIN A IN DAIRY CALF BLOOD PLASMA[1]

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Although the importance of vitamin A in the nutrition of the young calf is well recognized, present methods of evaluating the adequacy of dietary intake of this vitamin are not entirely satisfactory. One criterion commonly employed is the level of vitamin A in venous blood plasma or serum, estimation of which has been made primarily by colorimetric procedures. Most data reported in the literature were obtained by use of the Kimble method (7) which employs the Carr-Price color reaction and does not involve saponification. It has been demonstrated, however, that when this procedure is modified to include saponification prior to extraction with petroleum ether, higher vitamin A values usually are obtained (9).

Another colorimetric reagent which recently has been employed in the estimation of blood plasma vitamin A is activated glycerol dichlorohydrin (GDH). It has been observed (2), however, that among calves with apparently adequate dietary vitamin A, abnormally low blood plasma vitamin A levels occasionally were indicated when the Kimble extraction technique was followed by colorimetric assay with activated GDH. These low vitamin A values apparently were due to the presence in the petroleum ether extract (of nonsaponified plasma) of substances which suppressed the reaction between vitamin A and the colorimetric reagent, since saponification of plasma prior to extraction appeared to eliminate the inhibitory factor(s). Although there were individual calf differences, a relationship between diet and age and the presence of "inhibitors" in the blood plasma seemed possible.

The objectives of this investigation were to study factors which might be related to the occurrence of GDH-vitamin A color "inhibitors" in calf blood plasma, and to compare several lots of activated GDH in an effort to clarify occasional discrepancies between the nonsaponification and saponification methods of analysis.

APPARATUS

Beckman Model DU Spectrophotometer. This instrument was calibrated according to the directions given by the manufacturer. All spectrophotometric measurements were made with calibrated 1.00 cm Corex cells and with the slit-width setting maintained at 0.025 mm.

Water bath maintained at 60° ± 3°C.
Automatic pipette calibrated to deliver 4.0 ml of GDH (8).

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REAGENTS

95 per cent ethanol (USP).
Potassium hydroxide (85 per cent), reagent grade.
Skellysolve A, redistilled (b.p. 32-36°C).
Nitrogen, Linde.
Chloroform, reagent grade.
Activated glycerol dichlorohydrin (GDH).
USP vitamin A reference standard oil (vitamin A acetate in cottonseed oil).
β-carotene, crystalline, General Biochemicals, Inc.
Methanolic potassium hydroxide, 0.10 N.
Methanol, reagent grade.

EXPERIMENTAL AND RESULTS

Experiment 1. The reagents employed in this experiment were prepared routinely from GDH that had been used previously in analyses of vitamin A in blood plasma. Approximately 2 per cent antimony trichloride was added to the "used" GDH (containing about 20 per cent chloroform) and the chloroform fraction was removed by distillation under a partial vacuum. Subsequently the GDH was recovered by distillation at 10 to 40 mm pressure (Hg) and then was redistilled to give a clear, colorless and active material. Each lot of activated GDH was standardized against USP vitamin A reference standard oil and against crystalline β-carotene. The colorimetric characteristics of four lots of reagents employed in this part of the investigation are summarized in Table 1.

A rather broad survey was made of possible effects of breed, age and diet of calves on the presence of "inhibitors" in blood plasma. The animals, mostly Holsteins and Brown Swiss, were a part of the Iowa State College dairy herd and ranged in age from 4 days to 6 months, and were fed several different diets.

Table 1

Extinction coefficients of the reactions of activated GDH with vitamin A and with β-carotene

Reagent	$E^{1\%}_{1\ cm}$ after reaction with		
	Vitamin A	β-Carotene	
	555 mμ at 2 min.	555 mμ at 2 min.[a]	950 mμ at 4 min.
1	1132	139	2486
2	1150	152	2615
3	1115	137	2500
4	1239	130	2690

[a] Used in calculating carotene interference (11).

Samples of venous blood (potassium oxalate anticoagulant) were centrifuged and the plasma was analyzed for vitamin A and carotenoids both by nonsaponification and by saponification procedures (2). Analyses were made with a reagent until the supply was exhausted, then another lot was employed; a total of four lots of activated GDH was used.

Inspection of the data failed to indicate an effect of breed upon the presences of "inhibitors" in extracts of nonsaponified blood plasma. Although the age of the calves had no consistent effect on the presence of "inhibitors" there was a tendency to somewhat less inhibitory activity in plasma from older animals (4-6 mo.).

The data collected in this experiment are tabulated (Table 2) according to the reagent used and diet of the animals. Since in some instances more than one sample was taken from each calf, the "no. of samples" sometimes is greater than the "no. of calves". Although sample means were used in the present analyses, employment of calf means (using only the average value for each animal) did not alter the values significantly.

Table 2

Effects of diet and GDH reagent on the calf blood plasma vitamin A values obtained by nonsaponification and saponification methods

GDH Reagent	Diet	No. of Samples	No. of Calves	Mean Vitamin A Na/	Sb/	N/Sc/
				(γ/100 ml.)		(%)
1	Whole milk	4	2	1.50	13.75	10.9
	Filled milkd/ + vit. A	13	13	2.90	11.07	26.2
	Hay + conc.e/	24	9	4.64	17.75	26.1
2	Whole milk	14	9	14.91	18.25	82.7
	SMf/ + conc. + straw	15	7	8.21	10.65	77.1
	Filled milk + vit. A	2	2	12.70	14.60	87.0
	Hay + conc.	9	9	20.02	20.18	99.2
3	Whole milk	23	12	13.41	17.93	74.9
	SM + conc. + straw	9	7	11.46	10.67	107.4
	SM + conc. + straw + vit. A	22	7	13.32	14.59	91.4
	Filled milk + vit. A	2	2	10.85	14.10	76.9
	Hay + conc.	14	14	21.09	21.96	96.1
4	Whole milk	25	12	14.50	14.93	97.1
	SM + conc. + straw	38	22	10.05	10.14	99.0
	SM + conc. + straw + vit. A	58	17	13.52	13.82	97.8
	Filled milk + vit. A	8	8	12.85	13.98	91.8
	Hay + conc.	6	6	14.05	14.60	96.2

a/ nonsaponification method
b/ saponification method
c/ $\frac{\text{nonsaponification}}{\text{saponification}} \times 100$
d/ Reconstituted skimmilk with added fat dispersed by homogenization.
e/ concentrate mixture
f/ SM is a symbol for reconstituted skimmilk. This ration was a low-carotene diet used in producing vitamin A deficiencies in calves.

Table 3

Effect of GDH reagents on calf blood plasma vitamin A values

Calf No.	Reagent	Plasma Vitamin A	
		Non-saponified	Saponified
		(γ/100 ml.)	
3278	1	2.1	17.5
	2[a]	22.4	20.1
3282	1	1.5	12.8
	2[a]	16.4	17.8
3290	1	0.7	19.2
	2[a]	23.7	22.8
3291	1	2.0	11.9
	2[a]	17.4	17.3
3292	1	1.6	15.7
	2[a]	16.0	16.0
Av.	1	1.6	15.4
	2	19.2	18.8

[a] Sample drawn and analyzed 2 weeks following sample analyzed with reagent 1.

Within each lot of reagent tested, diet had no consistent effect upon the occurrence of color reaction "inhibitors". It also is evident from the data that the difference between the two methods of vitamin A determination was much greater with reagent 1 than with reagents 2, 3 and 4. This is illustrated further in Table 3 wherein it is apparent that there were marked differences between the nonsaponification values for blood plasma samples from the same calf which were analyzed with reagents 1 and 2. Blood samples used with reagent 2 were drawn approximately 2 weeks after those used with reagent 1. Insofar as possible, however, feeding and management practices were maintained constant. For each animal considerably less between-reagent difference was observed when the saponification procedure was employed. Although incomplete extraction of vitamin A might be responsible in part for the lower values in non-saponified plasma, this explanation seems inadequate in view of the large between-reagent differences.

Since the diet fed appeared to have no appreciable or consistent influence on the vitamin A values obtained by the two methods of analysis, the overall reagent effect on the vitamin A values was ascertained (Table 4). Analysis of variance (summarized in Table 5) of the calf plasma vitamin

Table 4

Summary of the mean blood plasma vitamin A values obtained with four lots of reagent

GDH Reagent	No. Calves	No. Samples	Mean Vitamin A Values		N/S[a/]
			Nonsaponified	Saponified	
			(γ/100 ml.)		(%)
1	17	41	3.75	15.24	24.6
2	24	51	11.58	13.61	85.1
3	34	102	12.60	14.51	86.8
4	37	127	12.34	12.65	99.3

a/ $\frac{\text{nonsaponification}}{\text{saponification}} \times 100$

Table 5

Summary of analyses of variance of vitamin A values obtained with four lots of reagent

Source of Variation	Reagent							
	1		2		3		4	
	df	Mean square	df	Mean square	df	Mean square	df	Mean square
Total	81		101		203		253	
Treatments (Saponification vs. non-saponification)	1	2707.68**	1	105.02**	1	188.12**	1	6.05*
Samples among calves	16	42.43*	23	137.05**	33	143.53**	36	38.81**
among samples within calves	24	19.64	27	51.58**	68	24.09**	90	19.61**
Error	40	21.93	50	7.11	101	5.23	126	1.11

** significant at P=0.01

* significant at P=0.05

A values observed in this experiment indicates differences significant at P = 0.01 between nonsaponification and saponification treatments with reagents 1, 2, and 3 and a difference significant at P = 0.05 with reagent 4. Although the mean difference between treatments with reagent 4 was small, the statistical data emphasize that the saponification values were quite uniformly higher than the corresponding nonsaponification figures.

The differences in blood plasma vitamin A among calves and among samples within calves were, with the exception of among samples with reagent 1, statistically significant at P = 0.01 (Table 5). These data served to emphasize the degree of variability among calves and among samples within calves even though the sampling occurred, in most cases, at approximately weekly intervals.

Experiment 2. The results of Experiment 1 suggest that breed, age, and dietary regime of the calves have comparatively little effect on the occurrence of GDH-vitamin A color reaction inhibitors in blood plasma, but that the characteristics of the GDH may be of major importance. Therefore, in Experiment 2, four lots of activated GDH which varied somewhat in chemical characteristics, method of preparation and period of storage prior to use were selected for study. Reagent 5 was prepared from glycerol (75% 2, 3-; 25% 1, 3-) dichlorohydrin supplied by the Shell Chemical Company. Prior to activation the GDH was distilled to give a clear, colorless and inactive product. Activation was then accomplished by the method of Sobel and Snow (11) using 1 per cent antimony trichloride followed by redistillation to give a clear reagent with a faint yellow tinge. This product is identified as reagent 5. A portion of this reagent was purified further by redistillation producing a clear, colorless liquid (reagent 6). Reagent 7 also was prepared from inactive "Shell" GDH using the same method as employed for reagent 5, but was prepared at an earlier date and was stored at 4°C for seven months prior to standardization, whereas the other reagents were standardized within one week after preparation. To allow comparison of these reagents (prepared directly from inactive GDH) with a freshly recovered and reactivated product, reagent 8 was prepared from previously used GDH (as described in Experiment 1) to serve the latter purpose. All reagents were standardized against USP vitamin A reference standard oil and against crystalline β-carotene. In addition, the free hydrogen chloride and antimony trichloride contents of these reagents were estimated. The former was measured by titration of 10 ml aliquots of reagent with 0.1 N methanolic potassium hydroxide using phenolphthalein as the indicator, and the latter was approximated from the ultraviolet absorption (at 230 mμ) of methanolic solutions of the reagents. Correction was made for the contribution of the antimony trichloride to the titration value. These data are shown in Table 6.

A solution of inactive GDH in methanol absorbs very little in the ultraviolet range, whereas, the absorption of a methanolic solution of GDH which contains antimony trichloride increases gradually with decreasing wavelengths. This absorption curve closely approximates that of antimony trichloride in hydrochloric acid reported by Whitney and Davidson (13), except for a shift to shorter wavelengths. The absorption at 230 mμ increases with increasing quantities of antimony trichloride, and thus an approximation of the quantity of this compound in the reagent is possible.

Table 6

Colorimetric characteristics and partial chemical composition of four lots of activated GDH

Reagent	N HCl	% $SbCl_3$	$E^{1\%}_{1\ cm}$ after reaction with		
			Vitamin A	β-Carotene	
			555 mμ at 2 min.	555 mμ at 2 min.[a]	950 mμ at 4 min.
5	0.0133	0.043	1143	142	3099
6	.0049	.020	1072	156	2801
7	.0134	.027	1137	130	3030
8	.0216	.032	1119	140	3174

[a] Used in calculating carotene interference.

The extinction coefficient ($E^{1\%}_{1\ cm}$) at 230 mμ of inactive GDH is 0.014, while that of antimony trichloride in the activated reagent is about 115.

Twenty-four calves, Holstein and Brown Swiss, ranging in age from 5 days to 9 weeks were selected for study. Since it was not practical to use all reagents on the blood from each calf, an incomplete block design, plan 11.1 described by Cochran and Cox (4), was employed to study the four reagents. With this design two reagents were tested on each calf. Therefore, six calves were required to test all pairs of the four reagents. This basic plan was repeated four times. In addition, a split-plot design was combined with the incomplete block design to compare the saponification and nonsaponification procedures (2) with each reagent. Duplicate determinations of the blood plasma vitamin A and carotenoids for each calf were made using both procedures with both reagents. Table 7 illustrates the experimental plan employed.

The mean blood plasma vitamin A and carotenoid levels as measured by saponification and nonsaponification methods are given in Tables 8 and 9, respectively. The values in each block are averages for blood plasma samples from four calves on which the same reagent was used. For reagents 6 and 7 the average vitamin A values found by the saponification method were uniformly higher than those indicated for the same blood plasma samples by the nonsaponification method, but for reagents 5 and 8 the averages for each method were essentially the same. On the other hand, for all reagents the average plasma carotenoid values obtained by the nonsaponification method consistently were slightly higher than those found by the saponification method.

Table 7 ·

Incomplete block design employed in experiment 2[a]

Calf	Treatment			
	Nonsaponification		Saponification	
	(Reagent)			
1	5	6	5	6
2	5	7	5	7
3	5	8	5	8
4	6	7	6	7
5	6	8	6	8
6	7	8	7	8

[a] This design was replicated four times, with a total of 24 calves.

The incomplete block design employed made it possible to combine the results obtained from all calves in such a way to eliminate the influence of calf differences from the reagent means. These adjusted means are summarized in Table 10. The analysis of variance of the data for both carotenoids and vitamin A is presented in Table 11. Reagents and saponification both influenced significantly ($P = 0.01$) the carotenoid levels and there was no indication of interaction between the two factors. For vitamin A the computed F's for reagents and saponification were at the 0.10 and 0.07 probability levels, respectively. Although the effects of these two factors are not significant at $P = 0.05$, the probabilities of the F's are too low to conclude that the factors had no effect.

DISCUSSION

Although little is known concerning the chemical nature of blood plasma substances which suppress the normal color reaction of vitamin A with certain lots of activated GDH, these "inhibitors" may be the same as, or similar to, the lipidelike factors (9) which interfere with the Carr-Price color reaction. Saponification prior to extraction of the plasma-alcohol mixture with petroleum ether appears to eliminate the effect of inhibitory substances in samples showing considerable GDH color-suppressing activity (2).

In Experiment 1, "inhibitors" were indicated in most of the nonsaponified blood plasma samples when reagent 1 was employed. With this reagent the N/S values $\left(\frac{\text{nonsaponification}}{\text{saponification}} \times 100\right)$ averaged only 24.6 per

Table 8

Mean blood plasma vitamin A values measured by non-saponification and saponification methods with four lots of activated GDH

Block[a]	Reagent 5		6		7		8	
	N[b]	S[c]	N	S	N	S	N	S
1	21.4	21.1	19.7	21.2				
2	15.3	14.7			14.7	15.1		
3	16.8	17.5					16.3	16.5
4			17.7	19.0	17.9	19.5		
5			20.1	21.1			21.5	21.8
6					18.9	20.2	19.8	19.8
Average	17.8	17.8	19.2	20.4	17.2	18.3	19.2	19.4
N/S[d] (%)	100.0		94.1		94.0		99.0	

[a] Each block represents samples from 4 calves.

[b] nonsaponification method

[c] saponification method

[d] $\frac{\text{nonsaponification}}{\text{saponification}} \times 100$

cent. For reagents 2, 3, and 4 these values were considerably higher, averaging 85.1, 86.8, and 99.3 per cent, respectively (Table 4). Even with these reagents, however, the differences between the nonsaponification and saponification values were statistically significant. It is recognized that these differences could be due in part to the extent of extraction of vitamin A by each method. Earlier work has demonstrated the presence of inhibitors in extracts of certain nonsaponified plasma samples (2).

Although the within-reagent variations indicate that there were differences in the amounts of suppressing agent(s) among the nonsaponified plasma samples, these results suggest that adequacy of the activation of GDH is a major factor in reducing the degree to which these inhibitors are manifest.

Penketh (10) suggested that the activating principle in GDH is free hydrogen chloride which is produced during the vacuum distillation with antimony trichloride. In Experiment 2 of the present study it was found

Table 9

Mean blood plasma carotenoid values measured by non-saponification and saponification methods with four lots of activated GDH

Block[a]	Reagent							
	5		6		7		8	
	N[b]	S[c]	N	S	N	S	N	S
1	16.2	14.9	17.4	17.0				
2	18.5	17.7			17.5	17.0		
3	12.8	11.6					12.1	11.3
4			8.4	7.8	7.2	6.5		
5			16.4	13.8			14.3	12.5
6					10.8	10.0	10.8	9.9
Av.	15.8	14.7	14.1	12.9	11.8	11.2	12.4	11.2
N/S[d] (%)	107.4		109.2		105.2		110.8	

[a] Each block represents samples from 4 calves.
[b] Nonsaponification method.
[c] Saponification method.
[d] $\frac{\text{nonsaponification}}{\text{saponification}} \times 100$

Table 10

Adjusted reagent means by treatments

GDH Reagent	No. Samples	Mean Vitamin A			Mean Carotenoids		
		Nonsapon.	Sapon.	Av.	Nonsapon.	Sapon	Av.
		(γ/100 ml.)			(γ/100 ml.)		
5	12	19.07	18.99	19.03	13.51	12.39	12.95
6	12	17.37	18.68	18.02	14.77	13.49	14.13
7	12	18.09	19.22	18.66	12.86	12.19	12.51
8	12	19.01	18.86	18.94	13.05	11.86	12.45
Av.		18.39	18.94		13.54	12.48	

Table 11

Summary of analysis of variance of vitamin A and carotenoid values in experiment 2

Source of Variation	Degrees of Freedom	Mean Squares	
		Carotenoids	Vitamin A
Repetitions	3	1992.96	384.27
Blocks, ignoring reagents	20	551.52	207.85
Reagent component	3	359.14	246.07
Blocks treated alike X reagents	15	579.84	211.42
Remainder	2	627.68	123.75
Reagents, eliminating blocks	3	19.36*	7.38[a]
Intra-block error	21	1.86	3.27
Saponification	1	54.93**	14.52[b]
Saponification X reagents	3	0.83	7.14
Sub-plate error	44	1.68	4.16
Duplicate determinations	96	0.55	0.54
Total	191		

*Significant at P = 0.05 ; **Significant at P = 0.01
a/ Probability = 0.10 ; b/ Probability = 0.07

that activated GDH which contains only traces of free hydrogen chloride and antimony trichloride (reagent 6) indicates a comparatively low N/S value (94.1 per cent) as compared to those values, 100.0 and 99.9 per cent, for reagents 5 and 8, respectively, which possessed larger quantities of antimony trichloride and/or free hydrogen chloride. Results when the latter reagents were employed indicate no significant differences between nonsaponification and saponification values. This suggests that certain minimum levels of free hydrogen chloride and antimony trichloride are required in activated GDH for greatest accuracy in the nonsaponification method of vitamin A analysis. It has been reported (3, 5) that a very sensitive reagent may be made by adding hydrogen chloride and antimony trichloride to GDH and also that saponification has no effect on the color reaction with this type of reagent.

Although the free hydrogen chloride and the antimony trichloride contents of reagent 7 were higher than those of reagent 6, the N/S values were similar. As previously indicated, reagent 7 had been refrigerated at 4°C for 7 months prior to standardization. This extended storage, although it had no apparent effect on the hydrogen chloride and antimony

Table 12

Effect of repeated redistillation on the hydrogen chloride and antimony trichloride contents and on the activity of GDH

Reagent	N HCl	% $SbCl_3$	$E^{1\%}_{1\ cm}$ (555 mμ)[a]
Original Activated GDH	0.0133	0.043	1143
After 1 redistillation	0.0049	0.020	1053
After 2 redistillations	0.0027	0.017	598
After 3 redistillations	0.0026	0.014	588

[a] 2 minutes after addition of GDH to a chloroform solution of vitamin A.

trichloride contents in the reagent or on the reagent activity as indicated by the color formed by reaction with vitamin A, may have enhanced deterioration of the activated GDH, resulting in a depression of the N/S values below those observed for reagents 5 and 8.

It has been suggested (12) that a reagent which is not clear and colorless should be redistilled to produce a suitable analytical reagent. It was found in the present investigation, however, that several redistillations of a reagent (employing the procedure of Sobel and Snow (10)) resulted in diminished reagent activity. Chemical analyses of the redistilled products indicated decreases in free hydrogen chloride and antimony trichloride and in activity with each successive distillation (Table 12), the most significant decrease following the second redistillation of the original reagent. The third redistillation affected little change in the antimony trichloride or hydrogen chloride contents or the activity of the reagent. It has been reported (1) that optimum activity of hydrogen chloride-activated GDH was obtained when the GDH was 0.01 M with respect to acid. Although the reagents employed in the present studies contained both antimony trichloride and hydrogen chloride, it is interesting to note that the level of hydrogen chloride in the reagent which showed no significant difference between saponification and nonsaponification procedures was near the optimum.

Kawasaki and Suenage (6) found that activated GDH gave different intensities of coloration upon reaction with vitamin A according to the method of preparation and activation. The present investigation confirms this, and in addition, suggests that the determination of free hydrogen chloride and antimony trichloride would aid in evaluating the acceptability of the reagent. It is apparent also that activated GDH which is less than approximately 0.01 N in hydrogen chloride and contains less than approximately 0.025 per cent antimony trichloride is of questionable value as an analytical reagent for the estimation of vitamin A in the blood plasma of dairy calves by the nonsaponification method.

The estimation of carotenoids in blood serum by activated GDH (11) was found to be equivalent in accuracy to the direct measurement in petroleum ether extract at 440 mμ. Each blood plasma sample employed in the present vitamin A study also was analyzed for carotenoids employing activated GDH and making the spectrophotometric measurement at 950 mμ. The latter wavelength was used because previous work (2) has shown that measurements at shorter wavelengths are less accurate.

Some between-treatment (nonsaponification versus saponification) differences in carotenoid values were noted. In Experiment 1 there was a tendency for the saponification carotenoid values to be somewhat higher than those observed by the nonsaponification method. The differences between treatments were greatest with reagent 1, suggesting a reagent effect similar to but not so marked as that observed in the vitamin A estimation. In Experiment 2 the carotenoid values were significantly higher by the nonsaponification method with each reagent. These results are not in accord with those in Experiment 1. The reason for this discrepance is not clear but it may be possible that some suspended particles could have been transferred with the extracts of the nonsaponified samples. In Experiment 1 a longer standing time, subsequent to pipetting, probably reduced the chance of error due to suspended material. This warrants further investigation.

In Experiment 2 when the carotenoid values for each reagent were adjusted to indicate reagent means (Table 10), it was found that the value for reagent 6 was significantly higher than those for reagents 5, 7, and 8, indicating that the use of a reagent deficient both in hydrogen chloride and antimony trichloride, which was shown to be unsuitable for the estimation of blood plasma vitamin A, also may result in unreliable blood plasma carotenoid values.

SUMMARY

The vitamin A and carotenoid values in calf blood plasma samples were estimated employing activated glycerol dichlorohydrin. The presence, in certain samples, of factors which suppress the normal colorimetric reaction of vitamin A with GDH was indicated. The effect of inhibitors in blood plasma was eliminated by saponification prior to extraction with petroleum ether.

Age, breed, and diet of the calves had no significant influence on the incidence or magnitude of inhibitory activity. The among-reagent differences, however, were rather striking.

It was found, employing an incomplete block experimental design, that reagents with less than approximately 0.025 per cent antimony trichloride and less than approximately 0.01 N in hydrogen chloride are not entirely satisfactory for estimating vitamin A in calf blood plasma even when the saponification procecure is employed. Repeated redistillation of GDH reduces both the free hydrogen chloride and antimony trichloride contents and renders the reagent unsatisfactory for vitamin A analysis. Results indicate further that the requirements for reliability of carotenoid estimations are similar to those for vitamin A.

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